

Antibiotic resistance: not only the clinician's problem.

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Antibiotics and the antibiotic resistance phenomenon in aquatic environments

Antibiotics are substances produced naturally by several microorganisms or synthesised (semi-) synthetically in the laboratory. They can either destroy susceptible microorganisms (bactericidal effect) or inhibit their development (bacteriostatic effect). They are one of the most important discoveries in human medicine, regarded as “magic bullets” that could target pathogenic microorganisms selectively, this leading to an intensive usage since more than 7 decades ago. Antibiotics were produced in natural microbial communities for millions of years but the „antibiotic era“ really began in the early 20th century. One of the major discoveries in the history of antibiotics is that of penicillin by Alexander Fleming in 1928, followed by its mass production after 1940. From there on started a golden era of antibiotics with several new classes and compounds discovered, having different modes of action: interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, inhibition of a metabolic pathway or disorganizing the cell membrane (Džidić et al. 2008; Gothwal and Shashidhar 2014). Antibiotics have saved millions of lives from deadly infections. However, their extensive use has led to the development of antibiotic resistance, rendering them ineffective against some pathogens. While the clinical side of this problem has been studied extensively, there is a less-studied environmental aspect.

With the increasing industrial production of antibiotics and their human and veterinary use these substances have started entering the environment due to incomplete metabolism in the organisms (between 25 % and 75 %), incomplete elimination during sewage treatment, land application of animal waste that contains antibiotics, improper disposal of unused or expired drugs etc. (Jjemba et al. 2006; Park et al. 2007; Zheng et al. 2011). Surface waters, groundwater, wastewater, soil, sludge, manure have been reported for contamination with antibiotics (Gothwal and Shashidhar 2014 and references therein). They are regarded as persistent or pseudo-persistent because the rate of entering the environment is higher than the rate of elimination, having direct and indirect consequences on microorganisms, plants, animals, and humans. The direct effects are the potential toxic effects such as affecting the chloroplast and mitochondrial protein synthesis in plants (Kummerer 2009), phytotoxic effects by causing chromosomal aberrations and by affecting the plastid division (Brain et al. 2008), inhibition of assimilation rate (Opriş et al. 2013). The major issue

of antibiotic release into the environment is their indirect effect of exerting a selective pressure in the environments they enter and modifying the metabolic activity of the microbiota towards selection of antibiotic-resistant mutants and the appearance of antimicrobial resistance phenomenon (Martinez 2009), with serious consequences on human and animal health. Antibiotic resistance is defined by the World Health Organization as the resistance to an antimicrobial drug that was originally effective for treatment of infections caused by it (www.who.int). Susceptible bacteria may become resistant to antibiotics through multiple and complex mechanisms, such as (i) exclusion of the antibiotic by the cell membrane; (ii) intracellular modification and/or deactivation of the antibiotic; (iii) reduction in sensitivity of the cellular target; (iv) extrusion from the cell; or (v) intracellular sequestration (Taylor et al. 2011). These mechanisms can evolve through mutation and selection (vertical transfer) or by acquiring resistance-encoding genes from other bacteria (horizontal gene transfer). In other words, a bacteria which does not present resistance to a certain antibiotic can pick up a resistance gene from another bacteria and therefore become resistant. The major concern is that human impact will increase the probability of transferring resistance genes into clinically relevant pathogens, the infections caused by them becoming harder and harder to treat.

Thus, because *antibiotics and antimicrobial resistance* genes have been detected in various compartments of the aquatic environment and in the soil and sediments (Stolker et al. 2004; Perret et al. 2006; Ye et al. 2007; Walsh 2013), and due to their effects, they *are getting attention as new classes of pollutants in the environment* (Martinez 2009; Yang et al. 2011; Gillings and Stokes 2012). From there they can recontaminate humans and animals, for example via drinking water, irrigation, soil, or foodstuff (*Fig. 1*).

Municipal wastewaters are considered to be major contributors to the environmental pollution with pharmaceuticals, antibiotic resistant bacteria and resistance genes (Göbel et al. 2004; Marti et al. 2013). Wastewater treatment plants use different methods to reduce the total number of bacteria, especially coliforms, in their final effluent, but the treatment is not efficient enough to remove antibiotic resistance genes from wastewaters, these being released into the receiving surface waters (Munir et al. 2011; Mokracka et al. 2012; Czekalski et al. 2014). *Hospital wastewaters* are also one of the most important factors contributing to the release of antibiotics and antibiotic resistance into the aquatic environment (Hocquet et al. 2016; Lien et al. 2016). In European acute care hospitals 20–30% of inpatients undergo an antibiotic treatment, thus the large amount of antimicrobials eliminated in the sewage system creates an ideal ecological niche for antibiotic resistant bacteria and

they facilitate the horizontal resistance gene transfer (Ansari et al. 2009). *Veterinary medicine and animal husbandry* are also a major source of environmental contamination with antibiotics and resistant microorganisms because the manure and slurry end up in the sewage or are either stored or immediately applied to the agricultural fields as fertilizers. The unmetabolized compounds present in the manure or their biologically active metabolites may move from the manure fertilized soil into groundwater and eventually surface water. Thus, antibiotics and especially resistant bacteria can spread by re-entering human and animal populations via drinking water, irrigation, soil or the food chain putting a serious threat on their wellbeing (Jerneja 2012).

The need for common efforts to tackle the antibiotic resistance problem

In the 2013 annual report on global risks, the World Economic Forum concluded that “[...] arguably the greatest risk to human health comes in the form of antibiotic-resistant bacteria” (Howell 2013). The spread of multiple antimicrobial-resistant pathogenic bacteria has been recognised by the World Organisation for Animal Health, the Food and Agriculture Organization, the World Health Organization (WHO) and other European organizations as a serious global human and animal health problem. For example, the European Centre for Disease Prevention and Control estimates that antimicrobial resistance results each year in 25 000 deaths and related costs of over € 1.5 billion in healthcare

expenses and productivity loss. A very recent report (O’Neill 2016) states very clear that if no joint action is taken, antibiotic resistance will be the first cause of death by 2050, being expected to claim more than 10 million lives each year worldwide and a cumulative cost of more than 100 trillion USD.

WHO developed the *Global Strategy for Containment of Antimicrobial Resistance*, with key recommendations to address the need for mitigating resistance that will hopefully lead to an increase of awareness on the antibiotic resistance problem, an improvement of antibiotic use in people and animal husbandry, increased resources for funding surveillance, research and education. Also, the European Parliament recognized that antimicrobial resistance is an important, largely unresolved, issue which has become a threat to public health in Europe and globally, resulting in longer, more complicated treatments, a diminution of quality of life, a greater risk of deaths, extra healthcare costs and productivity losses and adopted a resolution that calls for a further intensification of the fight against resistance to antimicrobial agents (EU resolution P7_TA(2011)0473). Overall, all the parties involved in the antimicrobial resistance issue recognize that excessive antibiotic usage is known to exert selective pressure on bacterial populations, that gene swapping among bacteria does occur, and that an expanding number of people, animals and animal products transverse the globe much more quickly than ever before. They underline the need of collaboration among stakeholders, clinicians, environmental microbiologists, policy makers etc.

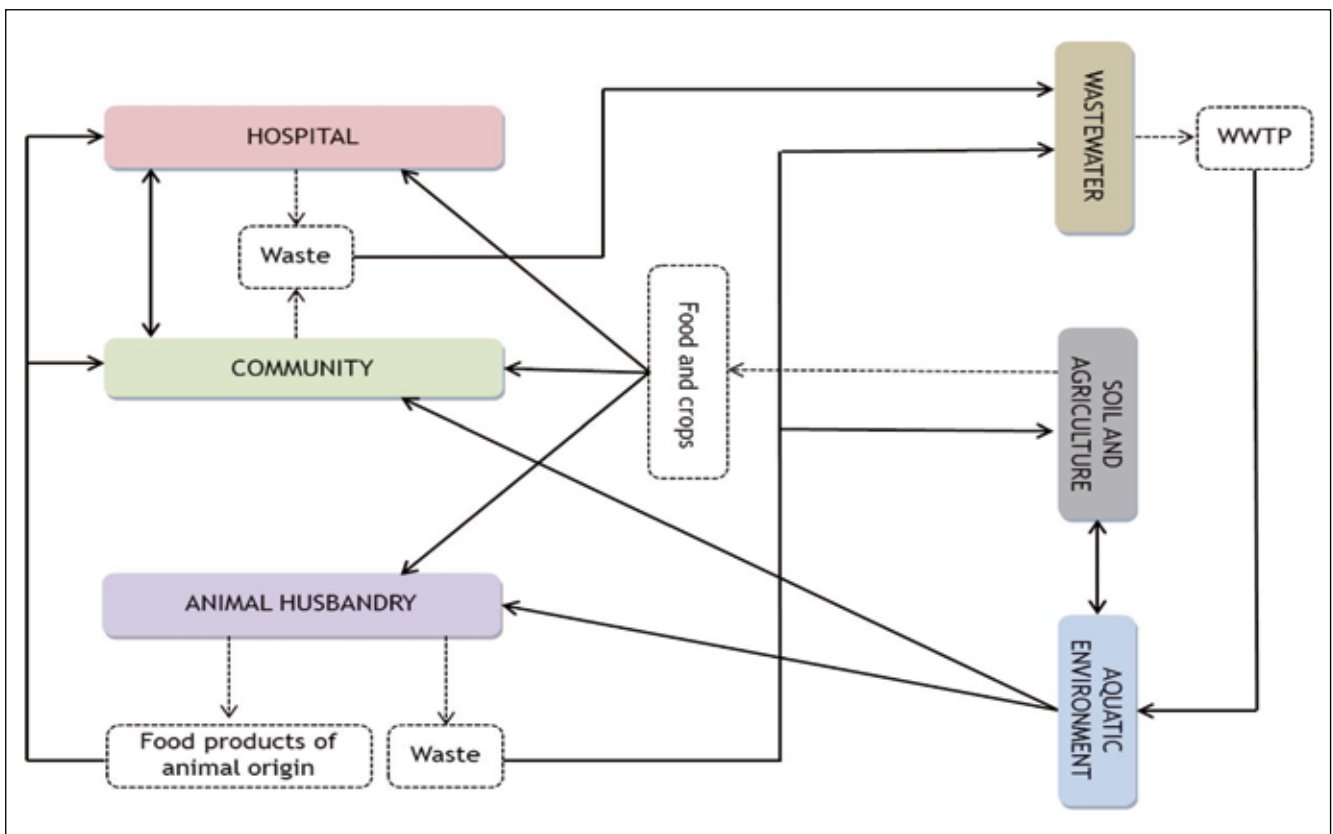


Figure 1. Schematic representation of major contributors to the spread of antibiotics and antibiotic resistance into the environment and possible routes for recontamination of humans and animals. Dash lines represent intermediary factors involved in the transfer. WWTP – wastewater treatment plant.

There has been considerable research effort to address the clinical ramifications of antimicrobial resistance and its mitigation. However, there remains a significant lack of information concerning the ecological risks from antimicrobial resistance present in the environment and the dissemination of causative agents, with limited regulations in place to mitigate these risks. It is more and more obvious that the environment plays an important role in the circuit of antibiotic resistance because it is either a natural source of antibiotic resistant bacteria and resistance genes (D'Costa et al. 2011) or it is receiving bacteria and genes released from human and animal husbandry activities that can spread across different ecological niches (Vaz-Moreira et al. 2014).

The antibiotic resistance in the Danube Delta – scratching the surface

The Institute of Biological Research Cluj-Napoca (ICB), branch of NIRDBS Bucharest, Romania is the coordinator of the EnviroAMR project – „Methodological guide for monitoring antibiotic residues and antimicrobial resistance in the environment as a supporting instrument for an enhanced quality management of surface waters and groundwater“, financed through the EEA 2009–2014 Financial Mechanism through the R004 – Reduction of hazardous substances programme. It is implemented in collaboration with the Biology and Geology Faculty from Babeş-Bolyai University Iuj-Napoca, with the Microbial diversity laboratory from the Norwegian University for Life Sciences and with the Nanostructured Systems Physics department from the National Institute for Research and Development for Isotopic and Molecular Technologies Cluj-Napoca. The general objective of the EnviroAMR project was to raise awareness in the matter of environmental pollution with antibiotics and the antimicrobial resistance phenomenon and to develop a state-of-the-art methodological guide for monitoring antibiotic residues and resistant microorganisms in the environment in order to design and implement efficient strategies for an enhanced quality management of surface waters and groundwater.

In May 2016, as part of the EnviroAMR project, project members took part in an extensive field trip in the Danube Delta due to a collaboration between ICB Cluj-Napoca (Dr. Cristian Coman) and the National Institute for Research and Development Danube Delta, Tulcea, Romania (Dr. Liliana Török). Eighteen lakes and six Danube points were sampled and a prescreening of antibiotic resistance genes was performed. Preliminary results show that several resistance genes are widespread in the Danube Delta underlining that microorganisms may present resistance to several classes of antibiotics (e.g. carbapenems, macrolides, aminoglycosides etc.). The most surprising fact is that mobile genetic elements shown to be actively involved in the horizontal antibiotic gene transfer are present in every sample investigated. As high abundance of these mobile genetic elements is linked to anthropogenic activity, it is important to perform

a thorough investigation of this phenomenon in order to design cross-border strategies and policies for a sustainable development of the Danube – Danube Delta – Black Sea axis. The methodological guide developed in the EnviroAMR project would be a suitable research framework in this matter because it proposes different methods to investigate the antibiotic resistance problem at different complexity levels (phenotype, genotype, metagenome).

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Key messages from a two year EU funded project for the future development of science and ecosystem management in aquatic ecosystems – the DANCERS Project

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This paper summarises the main outputs of the project DANube macroregion: Capacity building and Excellence in River Systems (basin, delta and sea)' (DANCERS) which aims at developing new instruments and tools for environmental research in the Danube Region.

The project objectives were to:

Critically analyse achievements in integrated river – delta – sea management in the Danube – Black Sea region placing them within the wider international context;

Identify strong and weak areas of regional environmental research;
 Derive a set of instruments to enhance environmental research and innovation in the region.

Since its start DANCERS has produced:

A Strategic Research and Innovation Agenda (SRIA) to 2020 and beyond;
 A concept and a detailed plan for two existing distributed research infrastructures in the region which can implement the SRIA;
 Proposals for an integrated educational programme (EDU).

Scientifically coherent solutions for integrated river-basin-sea management require an interdisciplinary approach that informs about decisions by society, industry and government. The SRIA addresses these research priorities in the context of the EU Strategy for the Danube.

Proposed Research Priorities for the Danube – Black Sea System

Restoring Ecosystem continuity throughout the DBS System

Two centuries of engineering work have heavily impacted ecosystems in the Danube River – Danube Delta – NW Black Sea continuum. Science must contribute to restoring longitudinal and lateral connectivity by implementing solutions to work with, rather than against, Nature.

Pathways of transport and accumulation of litter (especially plastic) and pollutants (including emerging pollutants) in the DBS System