Editorial

Bernd Cyffka: Editor DanubeNews, Applied Physical Geography, Catholic University of Eichstaett-Ingolstadt and Aueninstitut Neuburg/Donau, Germany, e-mail: bernd.cyffka@ku.de
Gertrud Haidvogl: Editor DanubeNews, Institute of Hydrobiology and Aquatic Ecosystem Management, University of Natural Resources and Life Sciences Vienna (BOKU), Austria, e-mail: gertrud.haidvogl@boku.ac.at

Dear readers,

As editors of Danube News, we are delighted to present in this volume the results of two scientific investigations of the Danube River and another project funded by the Danube Transnational Programme. Clemens Kittinger and his colleagues address the spread of antibiotic resistance in waterborne bacteria of the Danube. They summarize recent studies and focus in particular on knowledge gaps. Closing them in future research is without doubt a crucial contribution to environmental change and human health.

The article of Ionel Sorin Rîndaşu Beuran and colleagues offers insights to another project, which is currently funded by the Danube Transnational Programme. DANUBE FLOODPLAIN aims at linking flood protection and ecological needs of the Danube and selected tributaries. The project will develop tools and guidance to achieve long term solutions via floodplain restoration and conservation, while simultaneously decreasing flood risk. Wolfram Graf and his co-authors present a survey of macroinvertebrate fauna in instream structures of the Austrian Danube hydropower plant Freudenau, Vienna. Their findings have proven the role of such structures to the overall biodiversity of benthic macroinvertebrate fauna in impounded rivers. The volume closes with news and notes, among them announcements of recent publications as well as information on an open call for the funding price "Living Danube". We thank all authors for their exciting contributions!

State of knowledge on the spread of antibiotic resistance in the waterborne bacterial populations in the Danube: a mosaic with a lot of missing tiles

Clemens Kittinger: Medical University of Graz, Institute of Hygiene, Microbiology and Environmental Medicine, Graz, Austria, clemens.kittinger@medunigraz.at
Alexander Kirschner: Medical University Vienna, Institute for Hygiene and Applied Immunology, Vienna, Austria; Karl Landsteiner University for Health Sciences, Water Quality and Health, Krems, Austria; Interuniversity Cooperation Centre Water & Health, www.waterandhealth.at
Andreas Farnleitner: Technische Universität Wien, Institute of Chemical, Environmental and Bioscience Engineering, Vienna, Austria; Karl Landsteiner University for Health Sciences, Water Quality and Health, Krems, Austria; Interuniversity Cooperation Centre Water & Health, www.waterandhealth.at
Gernot Zarfel: Medical University of Graz, Institute of Hygiene, Microbiology and Environmental Medicine, Graz, Austria, Gernot.zarfel@medunigraz.at

The presence of human induced antibiotic resistance has become normal in aquatic environments

It is an unfortunate finding that the presence of human-induced antibiotic resistance in the environment can no longer be seen as an exceptional occurrence. Even in the most untouched areas of the world, this phenomenon can be found which did not exist there 20 years ago. Aquatic ecosystems are specifically affected by these developments. Humankind abuses them as a huge cesspool in which the most diverse leftovers of our civilization are disposed. This has initiated a selection process within the bacterial communities that can contribute in the long run to the blunting of one of the most important weapons against infectious diseases: antibiotics (Kummerer 2009, Livermore 2012). In reaction to this, the WHO has developed a global action plan on antimicrobial resistance (https://www.who.int/antimicrobial-resistance/global-action-plan/en/), for which a research agenda was set up in 2015 for water, sanitation and antimicrobial resistance, defining the need for "Identification and quantification of sources, occurrence, and transport of antimicrobial resistant bacteria (ARB), antimicrobial resistance genes (ARG) and antibiotic residues from humans and animals in the environment" (Wuijts et al. 2017). Most recently, the EU Antimicrobial Resistance (AMR) Action Plan has demanded concrete actions to close knowledge gaps on AMR in the environment to be implemented in all EU member states (European Commission 2018).

The specific knowledge of the input, the influencing factors and the exact mechanisms of the spread, accumulation and loss of antibiotic resistances in aquatic ecosystems is unfortunately still largely lacking. Mostly small-scale studies and short temporal courses form an increasingly dense network of mosaic stones, but the limitations of these studies are not compensated by their numbers. The network and plasticity of aquatic ecosystems (which do not end at national borders or estuaries) are the real challenge. Another challenge is the high genetic flexibility and genetic diversity within the affected bacterial species (and together with a very short reaction time the adaptation to treatment schemes) (Kummerer 2009, Fernandez-Astorga et al. 1992, Aminov & Mackie 2009, Juhas et al. 2009).

Therefore, persistence of these human-induced genes and bacteria varies from case to case. As a rule, it is not the resistance itself that is the decisive factor, but above all the host bacteria and the mobility of the genes between species, genera or across family borders (Fernandez-Astorga et al. 1992, Juhas et al. 2009).

Every bacterial species is telling a different story

Enterobacteriacaeae – This family plays a special role as carrier of antibiotic resistance in aquatic ecosystems. On the one hand, *E. coli* is one of the most common facultative pathogens, which has increasingly appeared in multi-resistant variants in recent years, and on the other hand it is an important water quality indicator. Therefore, it is not surprising that the data situation for this species is relatively good as far as the spread of resistances in water ecosystems is concerned. This focus may lead to comparability and a better database for estimating the spread of *E. coli,* but the question remains if the changes that occur in the *E. coli* population in rivers, lakes and the sea can be transferred to other bacterial species (Oliveira & Reygaert 2019, Bain et al. 2014).

Besides *E. coli,* the so-called ESKAPE *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter* species) are the most important germs with resistance problems. With the exception of *Staphylococcus aureus,* these are also species that can be found abundantly in the water ecosystem (Santajit & Indrawattana 2016). As far as the spread of resistance in the environment and in the water ecosystem is concerned, *E. coli* should be a good example for its genus and the family of *Enterobacteriaceae.* The exchange of resistance genes takes place easily via various genetically mobile elements and resistances that can be found in *E. coli* can also be detected in other species (Livermore 2012, Poirel et al. 2018). In contrast to many other *Enterobacteriaceae, E. coli* is not a typical environmental bacterial species. Nevertheless, dominant *E. coli* surface water isolates can be identified that differ from the dominant strains in healthy humans, animals or patient samples. For example, the strain MLST type ST131 should be highly

dominant in the extended spectrum beta lactamases (ESBL) forming *E. coli,* as it is in the human population. However, this type is found relatively rarely in water, especially in the vicinity of wastewater discharges. For example, only three of 17 isolates of *E. coli* ESBL isolates isolated in 2013 during the Joint Danube Survey (JDS3) could show this ST type. In other studies from tributaries their proportion was even lower (Kittinger et al. 2016, Zurfluh et al. 2013, Korzeniewska & Harnisz 2013).

In contrast to *E. coli,* the majority of *Klebsiella pneumoniae* environmental isolates found with ESBL resistance can be assigned to the same strains that dominate clinical isolates. Another difference to *E. coli* is that multi-resistance was more than five times higher in the *E. coli* isolates than in *Klebsiella* isolates from the River Danube (9.7 %) *(Figure 1).* This data is in concordance with other existing studies on resistance in *Klebsiella* isolates. Screening studies also showed a dominance of *E. coli* over the other *Enterobacteriaceae* (Kittinger et al. 2016, Poonia et al. 2014). A higher inflow of resistant *E.coli* from humans and animals may be a possible reason. It is interesting to note that the variance in the resistance distribution of *E. coli* over an entire river such as the River Danube is quite low. The proportion of multiresistant, resistant and nonresistant *E.coli* remains roughly the same from the upper reaches to the delta. However, at higher polluted sites and after discharge of wastewater, strains with extreme resistance behavior can be found which are not found in other sections or only very rarely. Nevertheless, their occurrence has hardly any influence on the total share of the multi-resistant population (Kittinger et al. 2016).

For other bacteria, the situation is probably very different. Also with *Klebsiella* one can find strains with many resistances primarily in the proximity of discharges. In contrast to *E. coli,* however, a different pattern of resistant *Klebsiella* can be observed along the course of the River Danube with the clearly highest proportion in the third section of the river *(fig. 1)* (Kittinger et al. 2016).

Pseudomonas spp. - In the case of *Pseudomonas* spp., the distribution of the strains with fewer human-induced resistances are found in the delta and those with very high rates in the upper course of the River Danube. *Pseudomonadaceae* also show physiological differences that lead to a different occurrence and spread of resistance in this family. In contrast to *E. coli* and many other *Enterobacteriaceae*, Pseudomonads have a very adaptive set of cell membrane associated efflux pumps that can easily mediate resistance to various toxic substances. By mutating these pumps, they can broaden this spectrum to get rid of other toxic substances at any time. Therefore, it is not surprising that Pseudomonads also choose this route of using efflux pumps as primary mechanism against toxins in surface water populations (Suzuki et al. 2013, Tummler et al. 2014, Kittinger et al. 2016). One example for this mechanism is the resistance to the most important class of last line anti-
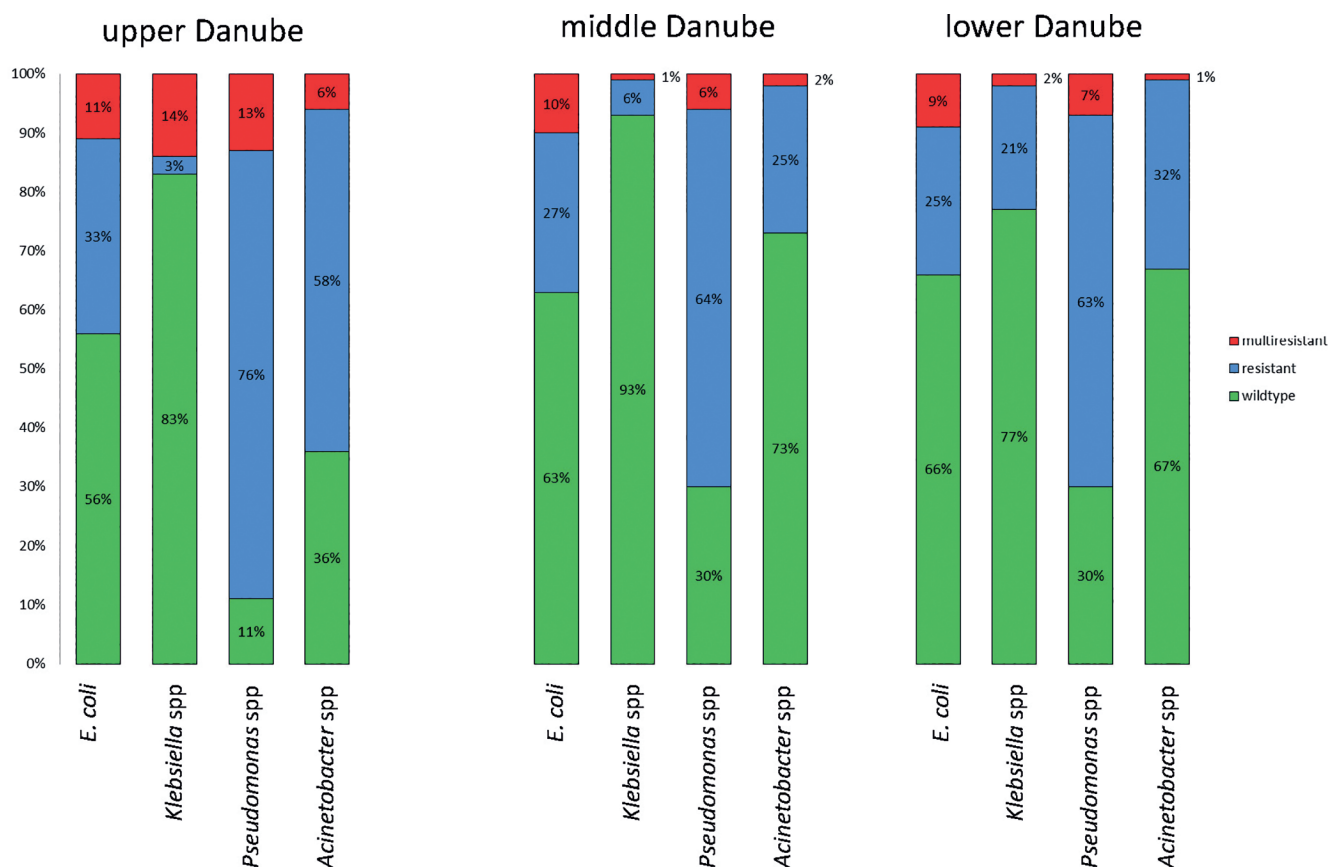
Figure 1. Comparison of populations of E. coli, Klebsiella spp, Pseudomonas spp and Acinetobacter spp. isolated from the same River Danube water samples (JDS3 2013). Graphs are divided according to wildtype resistance pattern, acquired resistance (resistance to 1–2 classes of antibiotics) and multi-resistance (3 or more classes).

biotics: the carbapenems. While the resistance of *Entero-bacteriaceae* isolated in the River Danube (but also in other river studies) to carbapenems is almost exclusively mediated by enzymatic destruction using special beta-lactamases, the situation in the pseudomonads is different. In less than 10 % of the carbapenem resistant Pseudomonads beta-lacta-mases are responsible for the resistance; the resistance is mostly mediated by efflux pumps. The isolates all over the course of the River Danube showed high resistance rates against meropenem (30.4 %) *(fig. 1).* Although the resistance against carbapenems in *Pseudomonas* spp. is mostly mediated via efflux pumps, carbapenem resistance poses a challenge for therapy, regardless of the underlying mechanism. For example, *Pseudomonas putida* are increasingly involved in hospital infections. These infections come up with severe complications and high mortality rates (up to 40 %). In most of these cases, multiresistant *Pseudomonas putida* were the reasons for the infection or nosocomial outbreaks (Kim et al. 2012, Kittinger et al. 2016). This dependency on efflux pumps has two consequences. Pseudomonas is not so reliant on the acquisition of new genetic elements and the broad range of substances affected by this pumps leads to development of resistance without the presence of specific antibiotics (co-selection).

Acinetobacter spp. – The genus *Acinetobacter* is a similar threat as *Pseudomonas* in hospital settings. It also has a high innate level of resistances against many antibiotic classes and is also an environmental species. The acquisition of antibiotic resistance over the course of the River Danube shows some similarities with the genus Pseudomonas, not when comparing total numbers of resistant bacteria but nevertheless in observable trends showing the highest numbers of resistant and multiresistant isolates in the upper section of the river (Oliveira & Reygaert 2019, Santajit & Indrawattana 2016, Kittinger et al. 2017) *(fig. 1).*

How to fill the gaps in the mosaic

– In most studies, either the culture method (which is restricted to a few species), or the detection at the DNA level (which does not allow species and genes to be assigned), are used as the only method, leading to a limited resolution of the antimicrobial resistance situation. Regarding mobile genetic elements, the drawbacks of using one method alone becomes even more apparent. In addition, focusing on a particularly closely examined species *(E. coli)* further increases the imbalance. In order to gain a better understanding of the

resistance situation in a large international river like the Danube, it is essential to understand the transfer mechanisms within the bacterial community. It is not only the presence or absence of resistance genes at the DNA level that must be investigated, but one also has to look for the reasons of the whereabouts and losses of resistance in the individual species. It is absolutely necessary to conduct these studies in parallel with isolates of different species in order to be able to identify the main influencing factors and to initiate countermeasures.

— As a basis for the understanding of sources, spread, accumulation and loss of antimicrobial resistance, reliable quantitative information must be available. So far, most studies provided only qualitative information on the presence or absence of ARB and ARG. Such quantitative data can also serve as a basis for the development of future guideline values demanded by health authorities.

— In order to develop effective management strategies, specifically the sources of antimicrobial resistances have to be identified. As input of ARB and ARG are most likely tightly linked to microbial fecal pollution, either of human (input primarily from municipal wastewater treatment plants) or animal origin (input primarily from agriculture and life-stock farming), the specific origin of fecal pollution has to be tracked with modern "microbial source tracking" tools.

— Moreover, only the comprehensive assessment of environmental conditions (the identification of sites where selection or co-selection for antimicrobial resistance may occur through e.g. heavy metals or pesticides) will enable a fundamental understanding of the mechanisms and importance of antimicrobial resistance in aquatic ecosystems.

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Danube countries review their options on flood risk management and include green infrastructures besides traditional measures in planning for a sustainable Danube

Ionel Sorin Rînda u Beuran: National Administration Romanian Water, Bucharest, Romania, e-mail: sorin.randasu@rowater.ro
Anemari Ciurea: National Administration Romanian Water, Bucharest, Romania, e-mail: anemari.ciurea@rowater.ro
Petrişor Mazilu: National Administration Romanian Water, Bucharest, Romania, e-mail: petrisor.mazilu@rowater.ro

Abstract

In compliance with the Flood Directive (FD) and Water Framework Directive (WFD), both the 1st Danube River Basin Management Plan (DRBMP) from 2009 (updated in 2015) and the 1st Flood Risk Management Plan for the Danube River Basin District (DFRMP) put forward ambitious targets