72 NRP
Antimicrobial Resistance
National Research Programme

ROUTES AND RESERVOIRS OF AMR-DETERMINANTS & ONE HEALTH AMR-SURVEILLANCE

Thematic Synthesis of
the National Research Programme
“Antimicrobial Resistance”

Swiss National
Science Foundation

November 2022

Contents

About this thematic synthesis

The National Research Programme “Antimicrobial Resistance” (NRP 72): Developing solutions to the threat of antibiotic resistance

Against the background of increasing antibiotic resistance, the Swiss National Science Foundation launched the National Research Programme "Antimicrobial Resistance" (NRP 72) on behalf of the Federal Council in 2017. In 33 projects at Swiss universities and higher education institutions, as well as 12 international projects within the framework of the European Joint Programming Initiative on Antimicrobial Resistance (JPIAMR), scientists investigated various aspects of the problem.

The aim of NRP 72 is to identify new solutions that contribute to the containment of antibiotic resistance. The programme was therefore planned and implemented in coordination with the Swiss Federal Strategy against Antibiotic Resistance (StAR).

Conclusions and recommendations for three overarching topics

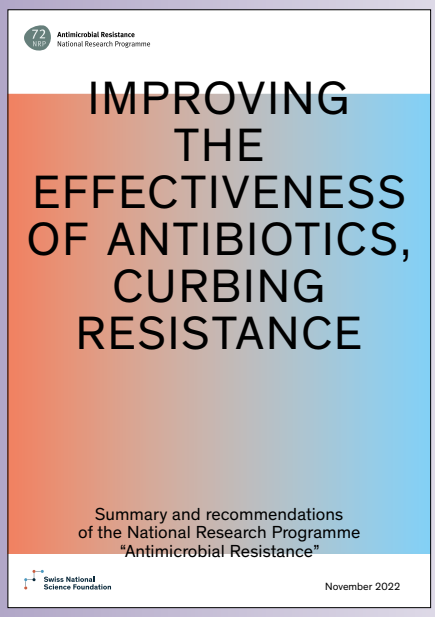
In addition, researchers synthesized their results on three overarching topics, corresponding to the three research modules of NRP 72:

- *Routes and reservoirs of AMR determinants & One Health AMR surveillance*
- *Optimized use of antibiotics and behavior changes*
- *Faster diagnostics and new therapeutic approaches*

For each topic, a working group of researchers analysed research results from different professional perspectives, put them in a larger context and discussed them with a sounding board representing relevant stakeholders as well as with members of the NRP 72 Steering Committee (see Annex 1 for involved persons). From these processes emerged three thematic syntheses, which elaborate scientific findings of NRP 72 and formulate recommendations for action.

The thematic syntheses stand on their own and reflect the view of the researchers’ working groups. However, together they form the central basis on which the NRP 72 Steering Committee derived its overarching conclusions on the most important fields of action and measures that result from the findings of the programme.

All three thematic syntheses and the overall programme summary can be consulted on www.nrp72.ch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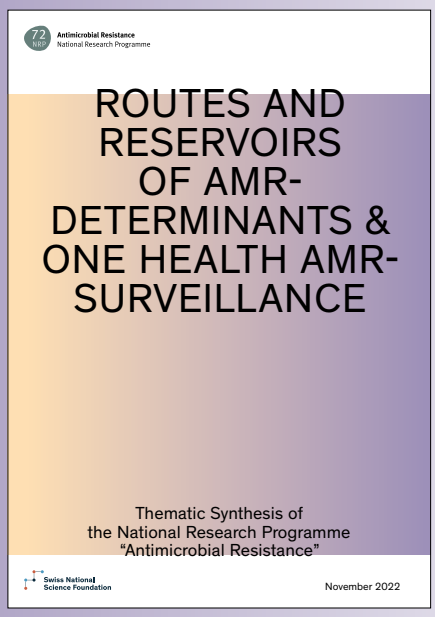
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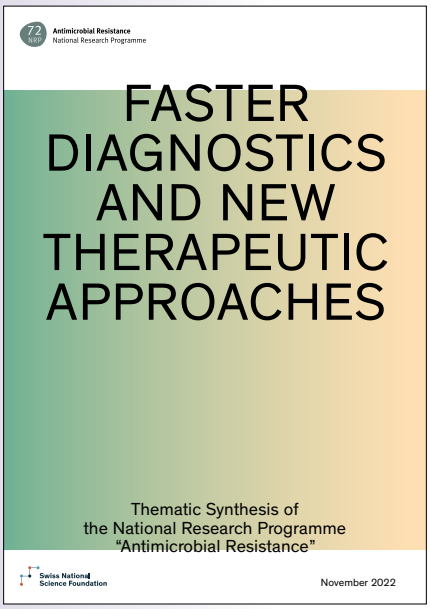
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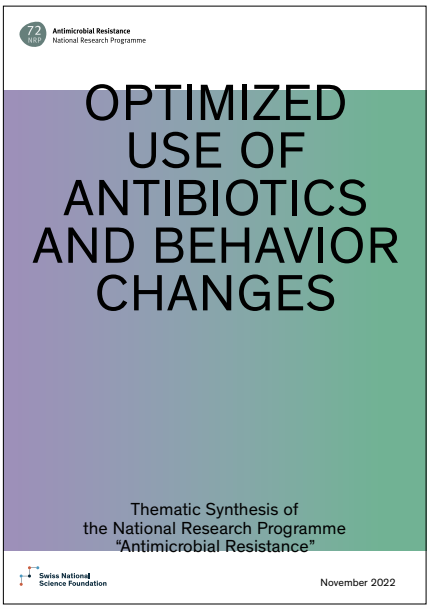
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Editorial

This is the thematic synthesis report for the NRP 72 Research Module 1 “Routes and reservoirs of AMR determinants & One Health AMR surveillance” and the associated JPIAMR (Joint Programming Initiative on Antimicrobial Resistance, a global collaborative organization for global coordination of AMR research) projects, whose Swiss contribution was funded by NRP 72.

The research summarized in this thematic synthesis was focused on answering various unsolved questions regarding the development and spread of AMR, and the relevant reservoirs of AMR in a One Health context. While research on the origins and spread of AMR is not new, many crucial knowledge gaps remained that impeded the design of successful intervention measures and policies to combat the accelerating spread of AMR. In addition, new technological advances, e.g., whole genome sequencing (WGS), provided new opportunities to explore AMR mechanisms, transmission pathways, and evolution with unprecedented molecular-epidemiological precision. Importantly, knowledge from non-medical settings, such as companion animals, aquatic environments, animal husbandry, and agriculture, was severely limited regarding links with resistance of clinical concern.

In this report we intend to present the progress made by Module 1 to interested stakeholders, decision makers, scientists, and the interested public, with the aim of making a difference in the fight against AMR. This thematic synthesis seeks to support the ongoing national and international efforts to combat AMR by summarizing the most relevant new findings from research, placing them in the context of current expert knowledge, and deriving recommendations for practice, policy, and future research.

In the final stages of the NRP 72 programme, a multidisciplinary working group of NRP 72 researchers was constituted for each research module with a mandate to integrate the individual projects and their results into a comprehensive synthesis report. The working group maintained close exchange with members of the NRP 72 Steering Committee and those responsible for NRP 72 management and knowledge transfer. Furthermore, in order to formulate recommendations for better monitoring and for improving control on the spread and emergence of AMR, a sounding board comprising reputed practitioners and experts representing key stakeholders and interest groups accompanied the working group. All these persons are listed in the publication details at the end of this report. They deserve great thanks for their valuable commitment.

The research presented in this report showed the potential of using WGS and metagenomics to obtain far deeper information on AMR on all levels, from antimicrobial resistance genes (ARG) and their association with mobile genetic elements (MGEs) to multi-drug resistant (MDR) pathogens. This made it possible to comprehensively explore and monitor resistance in diverse microbiomes or ecosystems. On this basis, NRP 72 made numerous contributions to the identification of new control points and surveillance opportunities for a true One Health approach to containing the spread of AMR.

Helmut Bürgmann
Coordinator of the NRP 72 Synthesis Working Group on “Routes and reservoirs of AMR determinants & One Health AMR surveillance”

Executive summary

New findings enable concrete measures at individual interfaces of AMR spread

The aim of the synthesis process on this topic was to derive recommendations from NRP 72 research that promote the implementation of new findings in practice. The focus of many projects was on the interfaces where antimicrobial resistance (AMR) can spread between humans, animals and the environment. In this One Health context, many research findings of NRP 72 provide the basis for concrete measures to interrupt or restrict transmission chains.

In addition to these concrete findings, it has also become apparent that the methods used in NRP 72 research are of great importance: It is a common feature of the projects presented in this thematic synthesis that they have applied new gene sequencing methods, such as whole genome sequencing (WGS), plasmid sequencing and metagenomics. These methods have developed very quickly in the last few years and are a prerequisite for the new insights presented here.

AMR surveillance across the whole One Health context is possible

But beyond insights into individual critical interfaces in the spread of AMR, sequencing methods also hold great potential for constantly and consistently monitoring AMR in a broader context that includes humans, animals and the environment. In principle, the detection of all resistance genes and their genomic context can provide precise insights into the spread of AMR, geographically and across the entire biological system. To exploit this huge potential, the data generated by new sequencing technologies must be brought together and combined with other data sources. NRP 72 researchers have developed a database on the basis of which this is possible. It lays the foundation for continuous seqeuncing-based AMR surveillance, including in One Health contexts. As a complement to existing surveillance systems, this new approach has the potential to enable faster and more targeted interventions in the future.

Four key recommendations for action

Based on the findings of NRP 72 research and in-depth exchange with numerous stakeholders, the working group "Routes and reservoirs of AMR determinants & One Health AMR surveillance" formulates a series of recommendations for action that take into account the specific situation in Switzerland. Briefly summarised, the following four key recommendations emerge:

- Whole genome sequencing (WGS) should be implemented as a standard surveillance tool.
- Screening and information campaigns for groups of people with increased likelihood of spreading AMR should be expanded.
- Monitoring of reservoirs outside the medical settings should be improved.
- Hygiene and control measures in veterinary clinics, agricultural production and food processing should be improved.

1

The One Health approach in the Swiss AMR strategy and in surveillance and research

Chapter summary

The challenges encountered in the field of routes and reservoirs of AMR determinants and One Health surveillance are multi-dimensional. The Swiss National Strategy on Antibiotic Resistance StAR takes this into account: from the outset, it has built on the One Health approach to combating the spread of AMR. Within its scope, the resistance situation is monitored in humans as well as in animals. Furthermore, Switzerland has – apart from NRP 72 – already invested considerably in research on AMR in the past and continues to do so at present. NRP 72 has thus built on previous research with the aim of addressing unanswered questions, and helping to steer and implement the national strategy.

One Health in the national strategy

The Swiss National Strategy on Antibiotic Resistance (StAR), adopted by the Federal Council on November 18th, 2015, coordinates and focuses on activities against antibiotic resistance. From the beginning, StAR has been built on the One Health approach (Schweizerische Eidgenossenschaft, 2015; link), acknowledging that antibiotic resistance is a public health problem where human health is intricately linked with the health and well-being of animals and the environment. All elements that underlie the antibiotic resistance crisis are linked across these sectors: overuse of antibiotics, evolution of resistance traits, their mobilization, horizontal transfer, and dissemination all occur within and across these sectors.

Nevertheless, the most severe problems with AMR are usually encountered in clinical settings. Efforts to push back against its spread have long focused on understanding and solving the problem in the medical area alone. Research in Switzerland, however, began years ago to also encompass farming and environmental aspects (NRP 49 Final Report; link). Thus, the work done in NRP 72 Module 1 could already build on a solid foundation and a framework for its task of studying routes and reservoirs of resistance dissemination in a One Health framework.

Resistance surveillance and the AMR situation

In terms of resistance surveillance, one of the key contributions of NRP 49 was the development of ANRESIS, the nationwide surveillance system and database for phenotypic antibiotic resistance and consumption (link). ANRESIS collects data on resistance prevalence and antibiotic consumption in human medicine. The IS-ABV (Informationssystem Antibiotika in der Veterinärmedizin) collects detailed information on antibiotic use and AMR monitoring in farm and companion animals and in meat (link). These data are published in the ARCH-Vet reports issued by the Federal Food Safety and Veterinary Office (FVO) (link). In 2022, an initial report on prescription of antibiotics for companion animals in Switzerland was published (link).

Thanks to ANRESIS and these reports, the current situation and trends regarding the prevalence of antibiotic resistance in clinical and veterinary settings is well documented, partially accessible by the public and summarized every two years in the Swiss Antibiotic Resistance Report (link). The reports reveal successes and failures in the efforts to combat antibiotic resistance to date. While, for example, Methicillin-Resistant *Staphylococcus aureus* (MRSA) rates have generally decreased over recent years, other resistances, such as fluoroquinolone and 3rd/4th-generation cephalosporin resistance in *E. coli* and Klebsiella pneumoniae have significantly increased. Important progress has been made in reducing antibiotics consumption in the veterinary sector, while consumption in acute care facilities and outpatient settings has until recently increased or stagnated, respectively (Swiss Antibiotic Resistance Report 2020, link). ANRESIS provides an important framework for scientific exchange and policy discussion in Switzerland. However, ANRESIS does not cover recent technological developments such as molecular epidemiology using sequencing.

Research on development and spread of AMR

Switzerland has an exceptionally strong research landscape to generate knowledge to address the challenges of AMR dissemination. Central to this strength are research centres at various cantonal universities and in the federal ETH domain. University hospitals and university teams directly associated with central infrastructures play a particularly important role as their research has a direct interface with medical practice. These include the National Reference Center for Emerging Antibiotic Resistance (NARA; link), associated with the University of Fribourg and the CHUV Lausanne; or the Swiss Centre for Antibiotic Resistance (ANRESIS, link), associated with the Institute of Infectious Diseases (IFIK, link) at the University of Bern. In addition, various reference centres on specific pathogens also focus on AMR-related surveillance functions and research. For the veterinary and food sectors, competence centres are found at the Vetsuisse faculties of the University of Bern and Zurich, the Center for Zoonoses, Animal Bacterial Diseases and Antimicrobial Resistance (ZOBA, link), and at the research institute Agroscope and other universities. Reference laboratories like ZOBA, NARA and a well-developed network of laboratories for human and veterinary medicine laboratories are interfacing with ANRESIS as a central phenotypic surveillance centre covering a large part of resistance data generated in routine diagnostics. In addition to these centres, research groups at universities across Switzerland also contribute substantially to this field of research.

A number of the aforementioned centres have also invested in research on AMR in environmental settings or study the interfaces of environmental and human and veterinary medical concerns. Environmental research on resistance is also performed by researchers at many Swiss Universities, universities, universities of applied science and research institutes like Agroscope and Eawag.

Switzerland has been committed to taking the AMR problem seriously for many years and has embraced research as a central element in combating the problem. This is evident from NRP 72 being the second National Research Programme on the topic. It is also evident from the central role given to research efforts in the national strategy against antibiotic resistance, StAR (link), as well as continued support for research through project grants from the Swiss National Science Foundation and National Centres of Competence in Research (NCCRs). In 2020, two NCCRs focused on microorganisms, including a focus on interaction between different compartments (human, animal, environment). These were NCCR Microbiomes (link) and NCCR AntiResist (link) (the latter being fully committed to AMR research).

2

Knowledge gaps and challenges for One Health AMR surveillance

Chapter summary

The One Health context is crucial – and highly complex

There are a multitude of societal, economic and scientific challenges that play a role in AMR. For this thematic synthesis, we focus on questions relating to routes, reservoirs, and surveillance arising from the multisectoral nature of the One Health perspective and from the complexity of the AMR problem itself. While the importance and the fundamental mechanisms of AMR spreading through vertical (transmission, proliferation, epidemiology), horizontal (gene transfer) and evolutionary mechanisms are already quite well understood, the complexity and combination of the issues, especially in a One Health context, still raise many crucial questions. The NRP 72 focused on challenges in understanding how resistances spread within complex microbial communities, within and between various environmental compartments, and ultimately to humans. The One Health perspective led to the realization that knowledge about many aspects of AMR spread was missing. Ascertaining which reservoirs of AMR contribute to infections with AMR bacteria crucially requires more knowledge about these reservoirs and routes of dissemination.

NRP 72 applied and explored new technologies to tackle open questions

The NRP 72 was also an answer to the challenge and opportunity that arose from the rapid progress in omics technologies over the last decade. Rapid sequencing of DNA, RNA and proteins on massive scales allow incredibly detailed views into the biological machinery underlying AMR. These technologies thus promise unprecedented new insights into how AMR evolves and spreads, and promise to revolutionize AMR research, but they also pose many new challenges regarding the analysis and interpretation of this data. Furthermore, question arise about how to fairly and ethically store, use, and share it, e.g., considering the FAIR (Findable, Accessible, Interoperable and Reusable) principles (Wilkinson et al. 2016). A shared platform for genomic data exchange and standardized analysis would considerably strengthen research on and surveillance of multidrug resistant pathogens. As a society, we are faced with the challenge of creating the necessary institutional and computational infrastructures that will be needed to integrate the new omics approaches into AMR surveillance.

The key research challenges that related to our thematic synthesis are to fill knowledge gaps regarding the emergence and spread of AMR and to integrate sequencing-based approaches into monitoring and surveillance of the spread of AMR; and thus to provide a basis for implementing monitoring and surveillance across all sectors relevant to the One Health approach.

The spread of antibiotic resistance

Antibiotic-resistant pathogens and antibiotic-resistance genes spread via human beings, animals and the environment, locally and globally. This close connection between the health of people and animals is therefore referred to as “One Health”.

2.1 Knowledge gaps regarding the emergence and spread of AMR

Considerable challenges remain in our understanding of the spread of AMR. The processes involved are very complex and play out on local and global scales (Frieri, Kumar, and Boutin 2017; Laxminarayan et al. 2013; Berendonk et al. 2015). On the one hand, they involve questions about 'vertical' spread of clones/strains. This involves epidemiological questions regarding dissemination through various routes of exposure and enrichments (e.g., through clinics, households, environmental systems, animals, and food). On the other hand, questions regarding resistance evolution via horizontal gene transfer (e.g., plasmids) or emergence of resistance through mutations play at least an equally important role. This multitude of strategies used by bacteria to spread AMR result in a constant need for us to update our knowledge.

Spread of resistant bacterial strains

There remain considerable gaps in our knowledge of transmission routes of antibiotic resistant strains. This is especially true for routes outside hospitals. Zoonosis (i.e. the transfer of AMR from animal to human) was probably the first environmental issue to receive attention, especially since large quantities of antibiotics were, and still are, used in animal husbandry (Muloi et al. 2018; Van Boeckel et al. 2015), including in Switzerland (Swiss Antibiotic Resistance Report 2020, link). Transfer to and from environmental reservoirs (e.g., the slaughterhouse environment; or the role of sewage, wastewater treatment and aquatic transport, manuring, irrigation, in the contamination of agricultural foodstuffs) are now recognized as important, but only partially understood pathways of AMR dissemination (Vaz-Moreira et al. 2019). Lack of knowledge of the links between environmental, animal, and human reservoirs remains a challenge. It has so far impeded quantitative attribution of risks of resistance acquisition from different reservoirs in the One Health continuum.

Emergence of new resistant strains and new resistance mechanisms

The emergence of MDR strains with new resistance mechanisms constitute constant major challenges both to human and veterinary medical practice, as the emergence and global spread of the plasmid-mediated transferable colistin resistance encoded by the *mcr-1* gene and its variants recently demonstrated (Arcilla et al. 2016). Because of these processes, AMR remains a moving target, requiring constant efforts by the research community and practitioners to remain a step ahead. A precise understanding of the processes involved in AMR evolution is essential for charting the best course of action.

Resistance evolution through horizontal gene transfer (HGT)

ARGs located on different mobile genetic elements (MGEs) such as plasmids and composite transposons can be disseminated in diverse bacterial species and settings through HGT (Huddleston 2014; von Wintersdorff et al. 2016). Integrons, important as gene-recruiting mechanisms, are often mobilized on transposons or integrated into other MGEs and thus able to spread by HGT (Gillings Michael et al. 2008). It is, however, also important to remember that the MGEs can also be integrated into the bacterial chromosome.

Acquisition of ARGs in pathogenic and environmental bacteria may occur by accidental events through co-selection, or, more frequently, under selective antibiotic pressure. Co-selection does not always include antibiotics; an influence, e.g., of disinfectants and metals, is known. Less clear is the selective potential of many other substances, e.g., pharmaceuticals or chemicals in personal care products. New MDR bacteria emerge and evolve by capturing and accumulating multiple ARGs on the MGEs, especially in high cell density aggregated conditions, which enables and promotes efficient HGT. For instance, in human and animal intes-

tines, HGT events easily occur due to i) high bacteria-to-bacteria contact, ii) availability of nutrient resources, and iii) exposure to antibiotics favouring selection and enrichment (Huddleston 2014). Moreover, long bacterial residence time in human/animal guts are ideal to permit HGT events through plasmid conjugation, transformation, and transduction by bacteriophages. However, other hot spots can also favour HGT between bacterial species (Smets and Barkay n.d.), e.g., in manured soil (Heuer, Schmitt, and Smalla 2011), aquatic biofilms (Abe, Nomura, and Suzuki 2020; Balcázar, Subirats, and Borrego 2015) and sediments (Marti, Variatza, and Balcazar 2014), and activated sludge in biological wastewater treatment (Li et al. 2018). However, the contribution of HGT in these environments to the greater picture of the emergence and transfer of AMR remains largely unknown.

Challenges also remain for our fundamental understanding of the relative importance of various HGT mechanisms. HGT mediated by plasmids or conjugative transposons is relatively well studied. In contrast, transformation (direct uptake and integration of exogenous genetic material) and transduction (gene transfer via phages) have received less attention in the context of their contribution to spreading and evolution of AMR (Keen Eric C. et al., n.d.; Balcazar 2014). This raises important questions regarding potentially overlooked pathways for the spread and emergence of resistance, be it in clinical or in environmental systems.

Resistance evolution through mutation(s)

Antibiotic resistance can be developed through the acquisition of an ARG or through mutation. Under antibiotic selective pressure, single or even multiple mutations can be selected and become dominant in the population. Usually, these mutations involve a mechanism of resistance that protects bacteria against the antibiotic family responsible for the specific selective pressure. However, there are examples of mutations triggering the expression of multidrug efflux pumps where a single mutation results in a multidrug resistance phenotype.

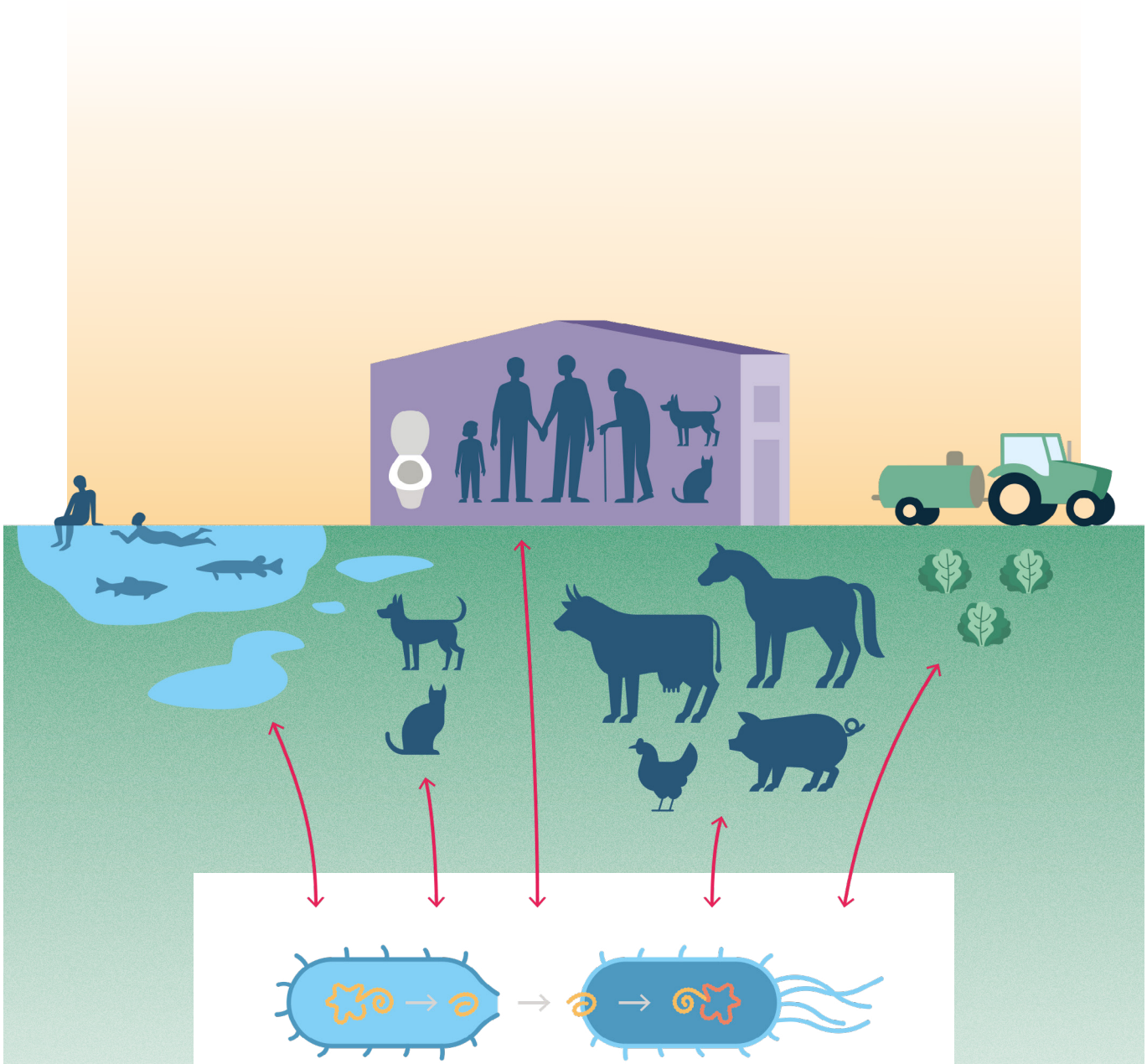
Well understood resistant mutations include modifications in the chromosomal genes encoding the targets and the transporters of the antibiotics. For instance, the use of fluoroquinolones can lead to selection of chromosomal mutations encoding for amino acid substitutions in the DNA gyrase and/or Topoisomerase IV (Bush et al. 2020). Once generated, this mechanism of resistance is vertically transmitted to the bacterial progeny. We note that fluoroquinolones are massively used in both human and veterinary (pets) settings, and these antibiotics can also "survive" for a long time in the environment (e.g., waters) (Czekalski et al. 2015; Wang et al. 2021).

In addition, ARGs can mutate too, which may enhance the activity and the substrate range of the encoded resistance determinants. These mutations can also involve extra-chromosomal ARGs. The best example is the evolution of the broad-/narrow-spectrum beta-lactamases into extended-spectrum beta-lactamases (ESBLs) able to hydrolyze last-generation cephalosporins. For instance, under the selective pressure of beta-lactams (the most used antibiotics), the plasmid-mediated narrow-spectrum TEM-1 enzyme evolved into TEM-type ESBLs with amino acid substitutions in positions 238 and/or 240 (Perez et al. 2007). Once evolved, these mechanisms of resistance could be transmitted vertically, but more importantly could also be transferred horizontally via MGEs to different bacterial species. Therefore, it would be important to better understand the epidemiological impact of mutations involving MGE-mediated ARGs in comparison to those only involving chromosomal mutations.

A further concern exists regarding the phenomenon of antibiotic tolerance, or persisters (i.e. the ability of a part of a bacterial population to persist in the presence of antibiotics without acquisition of a genetically encoded resistance or a change to the MIC of the population as a whole). So far there is insufficient knowledge about the role of persistence and how persisters may lead to the emergence of new resistant pathogen strains, especially in chronic infections (Kaldalu, Hauryliuk, and Tenson 2016; Moldoveanu, Rycroft, and Helaine 2021)

Complex dispersal through horizontal gene transfer

Antibiotic resistance spreads between human beings, animals and the environment. Many of these processes are hard to track, because different strains of bacteria can transmit antibiotic-resistance genes to each other. Even non-pathogenic bacteria thus participate in the spread of problematic types of resistance.



Because many different bacterial species transmit antibiotic resistance genes to each other through horizontal gene transfer, so-called reservoirs of resistance genes arise in the environment (e.g. in soil or water) and in human and animal hosts.

Challenges regarding the integration of sequencing-based approaches into AMR surveillance

Whole genome sequencing (WGS) has become the state-of-the-art technology for studying bacterial genomes. Beside the exploration of pathogen transmission by genome-to-genome comparison, the analysis of specific genes and the transfer of genes via MGEs is also possible (Schürch and van Schaik 2017; Köser, Ellington, and Peacock 2014). Genomes provide essential information about the ancestry of the isolate, evolution, and the resistance and virulence features. Using WGS data and state-of-the-art bioinformatics analysis allows a profound understanding of AMR. Therefore, integrating WGS into clinical practice, clinical and environmental monitoring, creating data platforms linking genomic and phenotypic data are critical elements in the fight against AMR. In addition, genomic data must be analyzed, visualized and shared between researchers. A key problem is the increasing amount of sequence data which is not available to researchers and public health experts. In particular, the FAIR principles of data sharing (Findable, Accessible, Interoperable and Reusable) are not fulfilled. A sequencing data exchange platform would allow sequencing data to be linked at the strain and gene level. Such a data sharing platform capable of handling large scale genomic data did not exist prior to the NRP 72 programme (Egli et al. 2018).

Metagenomics applies modern sequencing technologies to the study of microbial communities and environmental samples. Unbiased shotgun metagenomic data can be analyzed for their ARG content ("resistome") with bioinformatics tools based on ARG databases (Schmieder and Edwards 2012). While this allows a broad view of the ARG content of an environment, contextualizing this information in terms of the risk potential of environmental AMR is challenging (Martínez, Coque, and Baquero 2015): it is often difficult or impossible to determine whether the detected ARG actually confer phenotypic resistance, and if so, to what level. It is also difficult to identify the bacteria in which these ARG are located and whether they are located on plasmids or chromosomal. Furthermore, ways need to be found to link metagenomics information to WGS data from clinical or veterinary isolates. Technical solutions include long read sequencing, e.g., on the Oxford Nanopore or PacBio platforms and new bioinformatic platforms providing machine learning-based analysis. Standardizing methods and interpretations in the context of AMR monitoring and surveillance is yet another challenge (Bengtsson-Palme, Larsson, and Kristiansson 2017). Despite the challenges, metagenomics holds great promise in terms of expanding AMR research and monitoring into environmental contexts that make tackling these and other challenges worthwhile. Like WGS, metagenomic data also face the challenge of following FAIR principles on a common platform for data exchange.

The key questions are: what are the key requirements for sharing genomic and metadata information of pathogens across Switzerland? How can a sustainable environment be generated to share sequencing data for ARM surveillance?

2.2 Challenges regarding resistance monitoring in a One Health context

Important challenges with a focus on the One Health strategy exist regarding the exchange of information between the involved fields. Data exchange, in particular genomic data exchange, is critical. Current available systems (e.g., ANRESIS) focus on phenotypic data from routine diagnostics in human and increasingly also veterinary medicine, but do not provide access to genomic data and are not structured as a research platform to analyze and visualize WGS and metagenomic sequencing data. Improving monitoring/surveillance systems and databases in ways that facilitate linking observations across One Health will allow us to better measure the success of the overall efforts. Metadata provides additional contextual information, e.g., time and location of the sample from which a sequence originates. Even such simple information tremendously increases the value of the data by giving it a spatio-temporal resolution. Different fields in AMR surveillance (human, animal,

and environment) have specific wording, and an interoperable and controlled vocabulary is key to increase the efficacy on how different data types can be combined and holistically analyzed.

Surveillance is relatively well developed for human and veterinary medicine, and to some extent for food production. However, many aspects of the One Health view on AMR, as discussed above, e.g., within the environment, are not currently subject to systematic surveillance or monitoring. Surveillance of ARG distribution in hitherto neglected parts of the health system and in additional environments, like human/animal associated microbiomes, key terrestrial and aquatic environments and in animal husbandry and agriculture is therefore a logical first step in planning One Health approaches to understand the dynamics of AMR and to prevent the spread of new life-threatening ARGs. However, how to implement such surveillance and where additional surveillance or monitoring is most needed is still under discussion (Berendonk et al. 2015; Huijbers, Flach, and Larsson 2019; Vikesland et al. 2017).

The key questions are: Which systems should we monitor in the environment and with which methods? Which metadata are of particular interest in each field regarding overall One Health surveillance data management? How can researchers across all fields be motivated to use a single shared database across Switzerland for AMR surveillance?

Challenges in monitoring AMR in agricultural production, food production & industry

The food chain is an important link in the One Health context (Hernando-Amado et al. 2019; Perreten et al. 1997). The focus here is on the possible spread of AMR from various sources in food production (animals, farming, fermentation processes), food handling and transportation. In addition, many food items are traded globally, providing another route for international dissemination of AMR. The Federal Food Safety and Veterinary Office in collaboration with ZOBA monitors AMR and antibiotics use primarily in the veterinary context, e.g., in animal husbandry, and in meat products (link). In contrast to the human healthcare and veterinary sectors, AMR surveillance in food production and food products is not systematically recorded in public databases (e.g., ANRESIS, link). Systematic surveillance for other types of food does not currently take place. In addition, existing surveillance predominantly collects phenotypic data. WGS-based characterizations of bacterial isolates are provided in the context of studies by individual research groups, but the use of a standardized database, ideally containing metadata that may help to analyze the causes of emergence of antibiotic resistance, is missing.

Raw ready-to-eat foods (such as ready-to-eat raw plant-based foods) are important in view of the possible spread of AMR through the food chain, because there are fewer additional barriers to uptake (e.g., cooking) (Hölzel, Tetens, and Schwaiger 2018). Resistance transfer from environmental reservoirs (e.g., manure or river water used for irrigation) was therefore an important challenge for NRP 72 research. Currently, there are no requirements in terms of AMR (e.g., monitoring systems, risk-based product tests) for the use of river water as irrigation water or for manuring. However, there are ordinances and best practice guidelines for the use of organic fertilizers like manure that were developed with food hygiene in mind (Principles of Agricultural Crop Fertilization in Switzerland (PRIF), link). While these were not explicitly developed as measures against the dissemination of AMR, they do provide important safeguards for agricultural production in this respect. For example, they advise against the use of manure for vegetable production.

Possible intervention measures such as the decontamination of slaughtered animal carcasses, disinfection procedures for manure or wastewater, or the establishment of AMR process hygiene criteria for specific food categories have not yet been addressed, or may have to be re-evaluated as new evidence emerges.

Challenges in monitoring AMR in companion animals

The role of pets as reservoirs or vectors of AMR has recently received more attention (Pomba et al. 2017). Pets are among the animals with which humans have the closest contact as, many live in the home and are in daily contact with their owners. They are present in high numbers in Switzerland and often receive high-level medical attention, including antibiotic treatments. This indicates that zoonotic transfer of AMR from (and to) these animals could be a considerable risk. AMR in such animals or in small animal clinics in Switzerland has not been studied in detail. In contrast to the livestock sector, an AMR monitoring system has not yet been established for pets or animal clinics in Switzerland. Accordingly, the Swiss Antibiotic Resistance Report 2016 (link) reported little data on antibiotic use and resistance for pets. It is therefore an important challenge to better assess the AMR reservoirs and risks associated with this sector and, if necessary, propose improvements to AMR surveillance and veterinary practice.

Challenges in monitoring AMR in environmental systems

Monitoring of AMR in environmental systems poses unique challenges not addressed by clinical microbiology and therefore novel monitoring approaches should be established (Berendonk et al. 2015; Vikesland et al. 2017). The environments under consideration are highly diverse, and range from clinical surfaces to environments closely associated with humans like households or sewage, to agricultural environments and all the way to terrestrial and aquatic natural environments. The goals and targets for monitoring are accordingly diverse. In many cases, broader detection strategies are required as compared to the rather clearly defined analytical targets of an infection or an outbreak investigation in humans or animals. As both the purpose and setting of environmental research differ from human or veterinary medicine contexts, the standard bacterial isolation-based methods of clinical microbiology require considerable adaptation to derive meaningful results.

The goal of environmental research is frequently to obtain knowledge on the presence, distribution and possibly quantification of AMR, rather than to identify a specific causative pathogen and its susceptibility pattern. For this reason, methods that either provide this broad information or that are useful as indicators of general trends have to be explored. One such method is metagenomics, typically based on short read sequencing of environmental DNA (Schmieder and Edwards 2012). The metagenomic approach provides enormous opportunities, but also comes with its own specific set of challenges. Indicators, such as certain ARGs or indicator species that can be readily isolated, are a simpler approach and perhaps promising as a tool for large-scale environmental monitoring (Berendonk et al. 2015). However, universal consensus on the best indicator targets has not yet been reached. Where isolation-based strategies can be applied, WGS may provide an opportunity to connect AMR data from environmental and clinical contexts. Obtaining scientific information on whether environmental AMR monitoring may be necessary, and if so, where and how to best conduct such campaigns is therefore an important challenge for AMR research in Switzerland.

Challenges in identifying control and intervention points outside of the healthcare system

One of the key insights that led to the adoption of the One Health paradigm for AMR was that the traditional control and intervention points, such as infection prevention, clinical diagnosis, control programmes in healthcare settings, and adjustments to antibiotic therapy, appeared in many cases to be insufficient to stem the tide of increasing prevalence of AMR. While this approach has been successful in some cases (e.g., with MRSA), it was not effective in many others (especially regarding infectious agents with a wide distribution in communities, animals or the environment). Therefore, a current challenge is to determine where additional control points as well as new interventions are possible and useful.

Other challenges lie at the societal or political level. So far, the political or institutional will to expand AMR surveillance beyond the immediate needs of human and veterinary medicine is lacking. As no national or international efforts to monitor environmental resistance reservoirs have been launched, there are currently no standardized methods, nor have environmental limits or safety standards been established. The lack of systematic and sustainable activities in this direction is also due, at least in part, to the difficulty of formally establishing the risk associated with AMR circulating in environmental systems (i.e. a lack of quantitative attribution studies linking different sources and exposure pathways with clinical case data). Comparable to SARS-CoV-2 monitoring in sewage samples, a wastewater-based AMR surveillance system could be established. In Switzerland and most western countries with generally high environmental and food safety standards, environmental contamination rates by AMR organisms are likely low, while the contribution of environmental reservoirs to the long-term emergence or evolution of resistance is difficult to determine. In areas with lower standards of sanitation and food hygiene, the situation is likely to be different. Epidemiology shows that AMR is a truly global problem; therefore, Switzerland cannot afford to ignore what happens elsewhere. Again, data from environmental surveillance should also be shared within a common database, which would make it possible to compare the data to single strain information.

Challenges in assembling and ensuring accessibility of AMR surveillance data

The establishment of ANRESIS has been of enormous value for the efforts to constrain phenotypic AMR data, for medical practice and research. Nevertheless, challenges remain. One limitation of ANRESIS is that it collects phenotypic resistance data generated in routine diagnostics, and does not, for example, incorporate information from WGS, or metagenomic information from microbial consortia. The potential for surveillance and databases focused on genomic data has thus not been leveraged.

Also, while ANRESIS is comprehensive with regard to resistance in human and veterinary medicine, no monitoring programme or even structured database for resistance in the environment has so far been established. Thus, while the availability of information on organisms of concern in humans or from livestock is very good, knowledge of the AMR situation in pets, the whole food chain (except raw meat) and environmental reservoirs is more limited. Likewise, information on the transfer of resistance between these compartments and from these compartments to humans and livestock (i.e. attribution) remains more limited and less accessible (i.e. only through scientific literature).

While ANRESIS data is compatible with, and supplied to, the European surveillance effort of the ECDC (https://www.ecdc.europa.eu/en/antimicrobial-resistance), Swiss data is not integrated into the public databases and reports of the ECDC, complicating international comparisons. Swiss data of the veterinary field are integrated into ESVAC (AB use) and EFSA (AMR) reports. Furthermore, ANRESIS data is only partially open to the public (e.g., limiting public interactive data access to the last three years). The platform also does not allow full access for researchers. The FAIR data principle is not completely followed. The data at ANRESIS is not fully anonymized but encoded. In consideration of the HRA in the case of a research project it is not easy to exclude patient data from people who refused the general informed consent. The platform is designed as a monitoring tool for public health authorities, but not as a research tool. The amount of available metadata is limited and access restricted.

3

Scientific contributions of NRP 72: Routes and reservoirs of AMR and their surveillance

Chapter summary

Across 12 NRP 72 projects and 6 JPIAMR projects, substantial progress was achieved considering the previously described challenges. The projects generated a better understanding of reservoirs and routes of dissemination in Switzerland and provided a basis for further action in the fight against AMR.

An important development that cuts across a broad swath of the projects in this thematic synthesis is the application of sequencing approaches. A considerable number of projects have used this technology. This includes many studies applying WGS, which has been used broadly to characterize clinical and environmental isolates, to identify emerging resistance mechanisms or MDRO to track outbreaks and trace evolutionary origins. Another set of studies used metagenomic approaches for a wide variety of different purposes, from tracking resistance broadly in soil, water or on plants or for studying phages. Despite the great basis sequencing data provides to compare results across studies and across environments, even within the relatively close-knit research community within NRP 72, studies cutting across various projects are rare and limited. This highlights the enormous potential in another development of NRP 72: the development of the Swiss Pathogen Surveillance Platform (SPSP) as an important hub for sharing WGS and metagenomic data enriched with contextual meta-information for research and public health surveillance.

Another major achievement of the NRP 72 research discussed here is that it has broadly investigated hitherto neglected reservoirs and transmission pathways. NRP 72 studies provide a wealth of information on the resistance reservoirs in, e.g., soil, manure, plants and farms, in sewage, wastewater treatment plants and surface waters, as well as in neglected parts of the healthcare system, such as home care. The breadth of methods used is both a burden and a boon. It becomes clear in the synthesis of results that the data cannot yet be easily connected and used, e.g., for a universal attribution study for MDRO in Switzerland. On the other hand, the broad set of approaches and the enormously data-rich metagenomic approaches provide an excellent basis into monitoring strategies for so far unmonitored reservoirs and conduits that need to be included in One Health-oriented surveillance.

NRP 72 research has also contributed to fundamental new insights on AMR, e.g., on horizontal gene transfer mechanisms and evolutionary mechanisms that have received only limited attention so far but could be shown to impact the spread of AMR in new and often surprising ways.

The combination of foci on understudied environments and genome sequencing has ultimately led to important discoveries of emerging MDRO, or demonstrated uncommon outbreak mechanisms, e.g., at pet clinics including zoonotic transfer. Indeed, many projects have discovered new aspects of AMR risks that have directly contributed to the recommendations for action that will be discussed in Chapter 4.

The research results are subdivided into two sections. In the first, we focus on “Routes and Reservoirs of AMR determinants”. This section covers the scientific contributions focused on understanding the emergence, evolution and spread of AMR across all One Health sectors. In the second section, “One Health AMR-Surveillance”, we focus on the practical aspects of monitoring and surveillance of AMR for the One Health approach.

3.1 Routes and reservoirs of AMR determinants

3.1.1 Mechanisms of resistance spread and resistance evolution

Globally, the prevalence of antibiotic resistant pathogen strains is still rising (O'Neill Report, 2016; Van Boeckel et al. 2019). However, important progress towards reducing the prevalence of AMR pathogens has been achieved by dedicated measures, such as improved hospital hygiene (link) that resulted in the reduction of MRSA by reducing vertical transmission (link).

Mechanisms and importance of vertical transmission

The incidence of ESBL-producing Enterobacterales is still increasing in Switzerland (Swiss Antibiotic Resistance Report, link). This has therefore been addressed by NRP 72 projects.

During NRP 72, Profs. Endimiani and Perreten have shown for the first time that pandemic clones of *K. pneumoniae* (i.e., the OXA-48-producing ST11 and ST307) do similarly spread vertically in the veterinary clinics (Brilhante et al. 2021). Moreover, in the same veterinary context, additional carbapenemase producers (e.g., OXA-181- and NDM-5-producing *E. coli*) spread vertically with high frequency and can also be acquired by employees in contact with animals (Endimiani, Brilhante, et al. 2020; Nigg et al. 2019). In this overall outbreak situation, where the vertical spread was predominant, concurrent horizontal plasmid transmission and exchange among different Enterobacterales was also noted. For instance, IncL bla_{OXA-48}- and IncX3 $bla_{OXA-181}$-carrying plasmids were exchanged between *K. pneumoniae*, *E. coli*, and *Citrobacter* spp. at a veterinary institution (Nigg et al. 2019; Endimiani, Brilhante, et al. 2020). Overall, these findings demonstrated that the same countermeasures already implemented to limit the spread of Gram-negative superbugs in the human hospitals should also be implemented in the veterinary clinics (Schmidt et al. 2020).

Mechanisms of resistance mutation that contribute to emergent AMR

In the context of mutations involving MGE-mediated ARGs, a recent phenomenon of concern involving the spread of carbapenemase-producing Enterobacterales (CPE) should be mentioned (Poirel et al. 2020; Ramette et al. 2021). Avibactam is a new inhibitor of beta-lactamases, specifically designed to inhibit the KPC-type enzymes that confer resistance to all carbapenems (Endimiani, Choudhary, and Bonomo 2009), the last therapeutic options against MDR Gram-negatives. Although the combination of avibactam with ceftazidime was very promising (Endimiani et al. 2011), its massive and unregulated clinical use has rapidly generated mutations in the plasmid-mediated bla_{KPC} genes that encode for KPC variants with amino acid substitutions (e.g., in position 179) conferring resistance to ceftazidime-avibactam.

Under NRP 72, this phenomenon has also been described in Switzerland by the group led by Prof. Nordmann (e.g., KPC-41, KPC-50) (Mueller et al. 2019; Poirel et al. 2020). Since the plasmids carrying these ceftazidime/avibactam-resistant KPC variants are carried by MDR plasmids possessing other classes of ARGs, selective pressure generated by different kind of antibiotics (not necessarily carbapenems) can favour their survival and further spread in different settings.

Mechanisms and importance of horizontal AMR transfer

NRP 72 researchers reported that the same bla_{ESBL} (e.g., $bla_{CTX-M-1}$, $bla_{CTX-M-15}$) or bla_{CMY} pAmpCs (e.g., bla_{CMY-2} and bla_{CMY-42}) found in human isolates were also present in animal strains (Campos-Madueno et al. 2020;

Nigg et al. 2019); moreover, such plasmids were also very similar or even identical after WGS analysis. Therefore, this finding indicates the spread of common plasmids among different bacterial species and between diverse settings. Moreover, the importance of the horizontal transmission of MDR plasmids was also noted in two additional settings (i.e., waters and travellers). The mechanisms contributing to AMR plasmid transfer were further assessed *in vitro* and in gut infection models.

The group of Prof. Stephan, in collaboration with Endimiani and Perreten, showed that 10% of Swiss surface waters were contaminated with CPE (especially *E. coli*). Intriguingly, while the bacterial hosts were completely different to those described in the clinical context (i.e., different STs), most of these CPE carry plasmids (e.g., IncL $bla_{\text{OXA-48-}}$, IncX3 $bla_{\text{OXA-181-}}$, and IncQ $bla_{\text{KPC-2}}$-carrying) that were identical to those found in hospitalized humans and animals (Bleichenbacher et al. 2020). This supports the hypothesis that waters are an indicator of AMR pollution originating from highly selective settings (e.g., hospitals). Further work is needed to assess if such waters are the place where such transfer occurs, or if they are just indicators or plasmid sources for AMR plasmid transfer to environmental or clinically relevant strains in other sites.

The group of Dr. Bürgmann, approaching the topic for wastewater with metagenomic analysis, was able to show with a bioinformatics assembly-based approach that the degree of association of ARG with MGEs can be assessed from analysis of flanking regions of ARG-containing contigs. The proportion of mobilized ARG can be described by a 'mobility index'. They note that in wastewater treatment plants (WWTP) some ARG are nearly universally mobile according to this definition, including certain carbapenem resistance genes (Ju et al. 2019).

In collaboration with the Swiss Tropical and Public Health Institute (TPH) in Basel, Prof Endimiani's group has shown that Swiss travellers to Tanzania, not receiving antibiotic therapy, frequently become colonized (65%) at intestinal level with ESBL-producing and/or colistin-resistant *E. coli* strains (A. I. Moser, Kuenzli, Büdel, et al. 2021). Most of these MDR *E. coli* belonged to specific clones (e.g., ST361 producing CTX-M-15 ESBL) that were also carried by local healthy people and/or present in poultry, food chain, waters, and wild fish. This was a classic demonstration that the overall abundance in the environment of specific AMR organisms can contribute substantially to the vertical transmission of MDR bacteria to healthy people (i.e. the travellers). However, in a similar study conducted with Swiss travellers to Laos, a completely different phenomenon was observed (A. I. Moser, Kuenzli, Campos-Madueno, et al. 2021). In fact, 44% of the travellers returned colonized with MDR *E. coli*, but none of these strains was of the same STs (clone) as those detected in local people, animals, and environment. In contrast, the bacterial hosts carried the plasmids (e.g., IncX4 carrying mcr-1) that were identical to those identified in the *E. coli* spreading locally. It has remained unclear why AMR spreads via two different mechanisms in these two cases (i.e., vertical in Tanzania vs. horizontal in the Laos study). Strikingly, in these travellers, both types of AMR spread occurred in the absence of antibiotic therapies, suggesting that the acquisition/emergence of such MDR strains can be surprisingly efficient even without antibiotic-mediated selection. These observations call for further studies.

This phenomenon of the "double strategy" of AMR spread was also noted in a study performed by Prof. Endimiani's group in collaboration with ANRESIS involving ESBL-producing *Shigella sonnei* strains isolated in Switzerland (Campos-Madueno et al. 2020). Plasmids carrying $bla_{\text{CTX-Ms}}$ exhibited high degrees of genetic identity to each other, but also to plasmids previously reported in other Enterobacterales. However, there were also four main bacterial phylogenetic clusters, each of which included both ESBL-positive and ESBL-negative isolates. Moreover, matching isolates were reported in other countries. Overall, results suggested that some common *S. sonnei* clusters spread vertically between continents and can be imported into other nations after international trips. Such clusters include, in part, isolates that do not possess bla_{ESBL}-harboring plasmids, indicating their tendency to acquire them horizontally from other Enterobacterales.

Finally, the importance of the bacterial microbiota in the horizontal transmission of important plasmids has been explored. Prof Endimiani's group has shown that a fully susceptible *Salmonella* spp. strain causing infection in a patient suddenly became completely resistant to cephalosporins, making the treatment very challenging (Clément et al. 2019). The patient was coming from Sri Lanka and was colonized at the intestinal level with a Citrobacter amalonaticus carrying a conjugative IncFII plasmid expressing the DHA-1 pAmpCs.

Under the selective pressure of empirical treatment with ceftriaxone, rare *Salmonella* transconjugants which had received the AMR plasmid were apparently favored and replaced the non-resistant ancestral strain in that patient. This illustrates how antibiotic therapy further promotes the rise of AMR strains that emerged by HGT.

In the same context, Prof Endimiani's group has shown that a patient transferred from Macedonia (where he was hospitalized in the intensive care unit), imported into Switzerland (Bern) 5 carbapenemase-producing Gram-negative bacteria. In particular, 4 CPE (*Escherichia coli, Klebsiella pneumoniae, Providencia stuartii, Citrobacter sedlakii*) presented high-level resistance to all aminoglycosides due to the expression of the *armA* 16S rRNA methylase and possessed *bla*NDM-1 and *bla*OXA-48 carbapenemase genes. Overall, such life-threatening ARGs were carried in common IncC type plasmids, emphasizing the possibility of exchange *in vivo* and in the same patient of identical MGEs between different bacterial species (A. Moser et al. 2021).

The systematic analysis of such ESBL resistance plasmid transfer between different *E. coli* and *Salmonella enterica* strains has confirmed the efficiency of such transfer events *ex vivo* and in the intestinal ecosystem of mice (Benz et al. 2020). This work from the Bonhoeffer, Egli, Hall, and Hardt labs showed for several ESBL plasmids that this transfer is very efficient even in the absence of antibiotic selection. Here, the disruption of the microbiota either by previous exposure to antibiotics or by *Salmonella*-inflicted diarrheal disease, was a key driver. This fuelled *E. coli* and *Salmonella enterica* blooms, and the high donor and recipient densities explained the high rates of resistance plasmid transfer. Finally, the groups identified resistance plasmid reservoir formation in the host tissues of infected animals. These tissue reservoirs include a significant fraction of persisters, which are a special form that bacterial cells can take to survive immune responses and all types of antibiotics. These plasmid-carrying reservoirs of persisters are formed when invasive Enterobacterales (like *Salmonella enterica*) pick up plasmids and invade the host's gut tissue. The persister reservoirs can later re-seed the gut lumen and thereby enhance the chances of plasmid transfer to other Enterobacterales (Bakkeren, Diard, and Hardt 2020).

Based on our observations, we conclude that both vertical and horizontal transmission of AMR have a role in the One Health perspective, though the impact of these two mechanisms appears to differ among the different settings and the geographic areas assessed. This implies that only with the massive implementation of state-of-the-art molecular approaches (i.e., whole-genome sequencing) and the sharing of such data, the overall phenomenon of the AMR spread can be accurately studied, comprehended, and constantly monitored. We can also speculate that reducing the densities of Enterobacterales in any given environment may automatically reduce the chances for conjugative plasmid transfer, for which donors and potential recipients must meet physically. Thus, measures reducing Enterobacterales population densities (microbiota in manure, food and feed, infection-associated blooms etc.) could help to reduce not only vertical, but also horizontal spread of AMR. This aspect should be further investigated in the near future.

The contribution of transformation and transduction to AMR transfer

The research group led by Prof. Blokesch looked at the potential role of transformation in critically important resistant pathogens. In *Acinetobacter baumannii*, antibiotic resistance genes are often present on large genomic islands and some genes appear to be of extraneous origin (Blokesch 2017). The presence of several of the conserved natural competence genes was known, but transformation in *A. baumannii* had rarely been experimentally demonstrated at the onset of this study. The research of this project identified all conserved competence genes in a representative strain of *A. baumannii* and scored DNA uptake and recombination. Moreover, the role of a pilus that is central to the bacterium's DNA uptake machinery was studied in depth, which highlighted pronounced differences to the regulatory circuit and mechanistic aspects of this process in closely related non-pathogenic model species (Vesel and Blokesch 2021). *A. baumannii* was shown to be transformable at high rates, which, together with the genomic evidence, suggests that natural competence for transformation is an important mechanism of resistance acquisition in this organism. Supporting this finding is recent work that showed that natural competence is far more widespread than initially thought in this species,

especially in environmental isolates (Wilharm et al. 2017, Godeux et al. 2018, Hu et al. 2019). Collectively, these data indicate that natural transformation may be a horizontal gene transfer mechanism that not only fosters the acquisition of resistance genes from live AMR bacteria (Veening and Blokesch 2017) but also from DNA content that remains in environmental settings after the inactivation of resistant bacteria.

Dr. Gomez Sanz looked at the role of phages in the horizontal transfer of resistance. The work had to overcome considerable methodological challenges, e.g., isolating phages from various environments and correctly analyzing phage/viral metagenomes, and the methodological progress is itself a major contribution of this project. Her work clearly shows that wide host ranges and encapsulation of host DNA are common features of phages, underscoring their potential as HGT vectors (Göller et al. 2021; Göller et al. 2021). The abundance of ARG in environmental phages was low, but detectable, and the diversity of ARG was high. Overall, the results of this project indicate that transduction may not be a particularly important, but probably still non-negligible mode of HGT transfer of ARG.

3.1.2 Emerging species carrying important ARGs on novel MGEs

In the course of NRP 72, unusual and emerging bacterial species carrying ARGs on novel MGEs were described. Prof. Endimiani's lab reported the following findings: 1) a *Shewanella algae* strain co-harboring $bla_{\text{CTX-M-15}}$ and *armA* 16S rRNA methylase genes on a novel IncC plasmid (Endimiani, Bernasconi, et al. 2020); 2) a new $bla_{\text{VIM-1}}$-carrying IncN2 plasmid from an *Enterobacter hormaechei* subsp. *steigerwaltii* (Campos-Madueno, Gmuer, et al. 2021); 3) an XDR Proteus vulgaris isolate hosting a novel $bla_{\text{NDM-1}}$- and *armA*-carrying plasmid (A. I. Moser, Viaggi, Mauri, Carattoli, et al. 2021); 4) the first VIM-1 metallo-ß-lactamase-possessing *Klebsiella michiganensis* (Campos-Madueno, Sigrist, et al. 2021); 5) a cephalosporin-resistant *Comamonas kerstersii* strain carrying a novel class A beta-lactamase gene (A. I. Moser, Campos-Madueno, Keller, and Endimiani 2021); and 6) the global spread of *Klebsiella grimontii* isolates possessing $bla_{\text{VIM-1}}$ and *mcr-9* ARGs (Campos-Madueno, Moser, et al. 2021). In these works, core genomes of hosts were analyzed and linked with globally deposited genomes. Moreover, plasmid DNAs were independently assembled and compared with those reported worldwide.

The group led by Profs. Poirel and Nordmann also described the following key findings: 1) a miniature inverted transposable element at the origin of *mcr-5* gene acquisition in *E. coli* (Kieffer, Nordmann, et al. 2019); 2) the first genomic characterization of $bla_{\text{VIM-1}}$ and *mcr-9*-coharbouring *Enterobacter hormaechei* isolated from food of animal origin (Sadek et al. 2020); 3) the origin of *mcr-9*, an inducible gene encoding an acquired phosphoethanolamine transferase in *E. coli* (Kieffer, Royer, et al. 2019); 4) an MCR-like protein from *Kosakonia sacchari*, an environmental Enterobacterales (Fournier et al. 2021).

Overall, most of these findings underlined the importance of WGS vs. the MALDI-TOF MS for the correct identification of bacterial species. Furthermore, they emphasized the importance of having publicly available genomes to study (compare) the spread of novel species and/or MGEs at national and international level. For instance, two bla_{VIM}-positive *K. oxytoca* strains actually belonged to *K. grimontii* and were of ST172 and ST189. These two strains harbored IncHI2/HI2A and IncFII(Yp) plasmids carrying $bla_{\text{VIM-1}}$ together with *mcr-9* and $bla_{\text{VIM-1}}$, respectively. The IncHI2/HI2A plasmid showed itself to be identical to those carried by other Enterobacterales isolated from food and animal sources (e.g., Salmonella and Enterobacter spp. detected in Germany and Egypt) (Sadek et al. 2020). The IncFII(Yp) plasmid had a possible origin in Austria from an *Enterobacter hormaechei* carrying a highly similar plasmid. Core-genome phylogenies indicated that the ST172 *K. grimontii* belonged to a clone of identical Swedish and Swiss strains (≤15 SNVs to each other), whereas the ST189 strain was sporadic (Campos-Madueno, Moser, et al. 2021).

3.1.3 Insights into routes and direction of transmission of AMR-determinants across humans, animals, food chain, and environment

NRP 72 has helped to shed light on a number of important and previously underappreciated aspects of AMR transmission. Studies conducted within the NRP 72 have shed additional light on the role of transmission from environmental sources, or regarding the transport and persistence of resistant bacteria or resistance determinants in the environment.

As outlined in Chapter 2, knowledge on the environmental aspects of the One Health concept remains relatively underdeveloped. It is certainly justified that we give first priority to the systems most directly connected to the use of antibiotics, and immediately relevant to human health, i.e. medical practice, and second priority to the veterinary sector with comparable or higher use of antibiotics and a direct link to food production. However, the environment remains nonetheless an important reservoir, and its role as a source of AMR is, at present, understudied. Both immediate risks, i.e. those related to possible infection with resistant pathogens acquired in or through the environment, as well as longer term evolutionary risks (i.e. the acquisition and mobilization of new resistances from environmental reservoirs, or the recombination of resistance determinants in environmental systems) require our attention.

ESBL-producing Enterobacterales in environmental circulation

In her NRP 72 project, Prof. Tschudin Sutter aimed to unravel sources and transmission pathways of ESBL-producing Enterobacterales by analyses of detailed genome sequences obtained both from the sewage system and foodstuffs in Basel, as well as from clinical samples obtained at the Basel University Hospital. ESBL-producers were detected in more than 90% of all wastewater samples and comparisons between the distribution of ESBL genes, plasmid replicon types and bacterial strains revealed significant overlap with strains recovered from clinical samples. Food samples (chicken meat, salads, herbs, and sprouts) were contaminated with ESBL-producers in 9.4% of all cases. The distribution of ESBL genes and plasmid replicon types was less likely to overlap with the distribution seen in clinical and wastewater samples. Overall, a high diversity of ESBL producing *E. coli* and *K. pneumoniae* (the most commonly recovered species of Enterobacterales) was detected across all compartments, suggesting that they originate from genetically distinct sources rather than a few sources located within specific compartments. These analyses demonstrate the widespread dissemination of ESBL-producing Enterobacterales in the community (wastewater samples reflecting dissemination in the general population) and the medical relevance of strains circulating in the community due to the significant overlap with strains identified from clinical isolates of a tertiary academic care centre.

These findings indicated that transmission of ESBL-producing strains by foodstuffs to humans was likely rare in the studied settings in Switzerland, yet the detection of a unique cluster of ESBL-producing *K. pneumoniae* from a chicken sample and two clinical samples shows that the food chain may constitute a relevant transmission route for antimicrobial resistance genes (Aguilar-Bultet et al. 2020). Based on the results of a modelling study, human-to-human transmission within the open community alone may not be self-maintaining without transmission to and from nonhuman sources, underscoring the relevance of such findings (Mughini-Gras et al. 2019). Other studies have raised concerns over ESBL-producing Enterobacterales on vegetables imported to Switzerland (Zurfluh et al. 2015).

Research conducted in Swiss WWTP showed, using metagenomics and metatranscriptomics, that raw sewage transports a resistome (the identifiable ARG content of an environmental metagenome) that is closely related to the resistome found in the human gut (Ju et al. 2019). During the biological wastewater treatment process, this changes, indicating that the activated sludge microbiome contains a distinct resistome that is interacting with, and probably permeable to, the mobilized resistome of the human gut/sewage microbiome (Ju et al. 2019). The research also showed that while WWTPs typically remove >95% of ARG and AMR bacteria (Ju et al. 2019; Marano et al. 2020), some ARG appear to be overrepresented in the effluent com-

munity, and are also highly transcribed in this community, so that a relative enrichment of AMR in WWTPs (whether or not different treatment regimes may impact in a different way AMR relative abundance remains to be established) can at present not be ruled out. While the relevance of these findings regarding the risk of infection or AMR transfer from contact with the population via the downstream surface waters is currently still difficult to assess, there is also the question of the long-term consequences that come with providing a potential secondary evolutionary reactor, where AMR determinants from various environments, antibiotics and other selective agents, and large inputs of MGEs are mixed and can potentially recombine.

Short-read metagenomic data can be assembled and binned into draft genomes (Metagenome Assembled Genomes, MAGs) of abundant members of a community. Yuan et al. (2021) applied this approach to the dataset from Ju et al. (2019) and used it to characterize MDR hosts of (chromosomal) ARG. They found MAGs representing potential resistant pathogens, which remain present and transcriptionally active from influent to effluent of WWTPs. They also noted a high prevalence of ARG in denitrifying populations of the WWTP microbiome. Notably, this approach shows the potential of sequencing-based analysis to connect studies of AMR in complex microbiomes, which often pose difficulties in cultivation-based approaches, to WGS results from AMR isolates from clinical or veterinary surveillance.

In the “Swiss River Resistome” project, Dr. Bürgmann studied the further fate of AMR bacteria released with wastewater. The project expanded on earlier work by applying metagenomic approaches to obtain broad information on the ARG content of wastewater and wastewater-impacted aquatic environments. The results demonstrate the considerable impact of wastewater discharge on various indicators of AMR, as well as on a broad, metagenomic view of the resistome (Lee et al. 2021). This project provided information on the transport behaviour of AMR determinants released with wastewater into river systems. The study broke new ground by providing a full mass-flow analysis of AMR inputs, showing that the rapid decrease of AMR determinants over the first few kilometres was mostly due to dilution (Lee et al. 2021). However, the mass flow analysis, implemented by using various chemical substances released with wastewater as conservative tracers, also showed that loadings of various ARG experienced a relatively rapid decay during transport in the river through other processes (Lee et al. 2021).

In a separate study, the Swiss River Resistome project showed that wastewater bypass, i.e. combined sewage overflows that occur during stormwater events, temporarily lead to up to two orders of magnitude higher ARG levels in rivers than during normal WWTP operation, and thus to temporarily significantly increased potential exposure risks (Lee, Beck, and Bürgmann 2022).

Resistance spread and reservoirs in agricultural production

Further projects within NRP 72 investigated the potential role of the food chain in entertaining (vertical) transmission of antibiotic resistant bacteria. Dr. Jörg Hummerjohann found clinically relevant ESBL producers to persist in soil and lettuce after introduction by manure or water, pointing to the ability of such strains to survive in the environment (Gekenidis et al. 2020), thus pointing to an important potential reservoir. Dr. Markus Hilty’s longitudinal study provided insights regarding antibiotic resistance in pig farms (Moor, Aebi, et al. 2021). Piglets and pig farmers' stools and their metadata along with environmental samples were studied. As a result, working on the pig farms was not associated with an increased prevalence of MDR *E. coli* strains in these people. Clonal spread and horizontal gene transfer within pigs, but not between humans and animals, was detected. Liquid manure was identified as the major environmental reservoir in the farm environment. Pig farming practices like all-in-all-out systems that allow high hygienic standards, but not antimicrobial usage, were associated with reduced risk of MDR *E. coli* carriage at farm level. As carriage duration was normally short within the individual pigs, the risk of recolonization and clonal spread of MDR *E. coli* might be reduced by applying appropriate decontamination strategies. Of note, using 16S rRNA gene sequencing and the DADA2 pipeline, the same group showed that pig workers' stool samples shared fractions of the microbiota with the samples from their pigs (Moor, Wüthrich, et al. 2021).

The JPIAMR Project of Prof. Van Boeckel studied AMR on pig farms in Thailand (Huber et al. 2021). Their work shows that in Thailand, and probably in many other countries where regulations on antibiotic use for farming are loose, activity is urgently needed to avoid an accelerating development of AMR. Farms that have intensified their production and those that have geographically easier access to antibiotics (distance to drugstores) had elevated levels of AMR. The researchers did not find evidence of transfer of AMR from pigs to farmers, however. AMR surveillance and education of farmers will be important in reducing problems as agriculture transitions to more intensive farming methods.

Resistance spread through travel, gut microbiome colonization

As mentioned above, the group led by Prof. Endimiani has shown that many travellers visiting low-/medium-income countries with high AMR prevalence and sometimes with sub-optimal sanitization programmes, return to Switzerland colonized at intestinal level with ESBL-producing *E. coli* strains (e.g., 45–65% of those visiting Tanzania or Laos) (A. I. Moser, Kuenzli, Büdel, et al. 2021; A. I. Moser, Kuenzli, Campos-Madueno, et al. 2021). Molecular analyses clearly demonstrated that some local sources (e.g., people in the community, food chain, water, and food producing animals) are responsible for the transmission of these MDR bacteria to travellers. This transmission can occur using vertical (clonal) or horizontal (plasmid-mediated) strategies (A. I. Moser, Kuenzli, Büdel, et al. 2021; A. I. Moser, Kuenzli, Campos-Madueno, et al. 2021). More importantly, these individuals are at risk of developing future difficult-to-treat infections due to MDR *E. coli* and/or may transmit these bacteria to other people in close contact with them (e.g., in the Swiss household). Therefore, it is essential to decipher the impact and key factors favouring intestinal colonization and develop effective strategies to decolonize returning travellers to prevent transmission and limit the spread of "superbugs" in countries that still have a low prevalence (e.g., Switzerland) (A. I. Moser, Kuenzli, Campos-Madueno, et al. 2021).

In this context it is significant that a survey conducted by Prof. Harbarth indicated that admission screening for MDRO in Swiss clinics is not universally performed (28% of private and 9% of public institutions did not perform any screening) and heterogeneous with regards to the organisms covered and the procedures applied. The difficulty of identifying high-risk patients was frequently named as an impediment to admission screening (Martischang et al. 2019).

In addition to travellers, patients returning from hospitals might represent a source of transmission. As mentioned, Prof. Endimiani's group has shown that a single patient hospitalized abroad can remain colonized with 5 different carbapenemase-producing Gram-negatives and import them in Switzerland (A. Moser et al. 2021). More importantly, the same research group has shown that a Swiss patient hospitalized in India due to SARS-CoV-2 B.1.617.2 lineage (VOC Delta) infection returned to Switzerland colonized with a ST410 carbapenemase-producing *E. coli*. In particular, the strain carried a new plasmid-mediated variant of OXA-48 (i.e., OXA-484). This finding strongly underlines that patients hospitalized abroad can contribute to the importation of new carbapenemase producers (PMID: 34718203).

A two-year multicentric prospective study by Prof. Harbarth and colleagues at the University of Geneva (Martischang et al. 2020) followed carriers of ESBL-producing *E.coli* and *K.pneumoniae* returning from hospital and their household members with the goal of quantifying acquisition and transmission events using WGS. Seventy-one previously hospitalized patients colonized with ESBL-producing *E. coli* (n = 45), *K. pneumoniae* (n = 20) or both (n = 6) were included in the study after hospital discharge. All participants (71 previously hospitalized patients and 102 household contacts) were monitored for 4 months with the collection of fecal samples. The acquisition rate of ESBL-producing bacteria among previously negative household members was 1.9 per 100 participant-weeks at risk. Nineteen clonally related household transmissions were measured, translating to a transmission rate of 1.18 transmissions per 100 participant-weeks at risk. The identified risk factors included providing assistance for urinary and fecal excretion to the hospital-discharged persons. Essentially all transmissions took place during the first two months following hospital discharge (Riccio et al. 2021). This study highlights the importance of hygiene measures in community settings and could support

behavioural interventions to better control transmission of bacterial resistances in the community. A useful implementation would be 1) to inform patients that they are carriers of ESBL-producing bacteria at hospital discharge (which is currently not the regular practice), and 2) to hand out an information sheet to family or other household members explaining the improved hygiene precautions that should be taken.

In this context, new *in vitro* and *in vivo* models to study the dynamics of gut microbiota are needed. Prof. Lacroix set up an *in vitro* chicken continuous cecum fermentation model to study the effects of a CTX-M-1-producing *E. coli* or a vancomycin-resistant *E. faecium* on the bacterial populations of the intestinal tract under antibiotic selective pressure (e.g., cefotaxime) (Lacroix, Constancias, Greppi, unpublished data). Such *in-vitro* models will be valuable tools as they allow methodical study of processes like invasion of a microbiome and HGT of ARGs and could be used also to study possible interventions without animal models or studies in humans. The Lacroix group, for instance, is conducting research on reuterin and glycerol as compounds that may reduce the abundance of MDR Enterobacterales in the chicken cecum model.

Using a similar model, Prof. Endimiani's group investigated the use of bacteriophages as a strategy to decolonize intestinal carriers of CTX-M-15-producing ST131 *E. coli*. Results indicated that bacteriophage cocktails may be implemented to decolonize some intestinal carriers. However, the individual microbiota composition may have an impact on the development of phage resistance. Mechanisms underlying this phenomenon are likely to be diverse and complex and should be studied in the near future (Bernasconi et al. 2020).

Role of tolerance in the evolution of new AMR mutations

The laboratory of Prof. Jenal studied the role of antibiotic tolerance in promoting ARM development. Tolerance refers to the phenomenon of reduced killing kinetics by bactericidal antibiotics, while the MIC does not change. This can affect entire populations of pathogens or be limited to a subfraction of the population called persisters. This mechanism may provide previously unrecognized pathways that reduce antibiotic therapy efficiency and that may promote evolution of resistance, specifically in chronic infections.

Using chronic *P. aeruginosa* infections as a model, *in vitro* evolution experiments and the analysis of isolates from chronically infected Cystic Fibrosis (CF) patients indicated that tolerance is an important phenomenon that facilitates the evolution of new antibiotic resistance mutations (Santi et al. 2021). Similar observations were made by other labs studying clinical *S. aureus* infections. The evidence suggests that improved antibiotic treatment regimens may be able to slow or suppress the emergence of resistant mutants during chemotherapy (Liu et al. 2020).

The collaborative study by Bakkeren et al., which involved multiple NRP 72 experimentalists and modelers, further showed that persister subpopulations of *Salmonella enterica* promote the spread of antibiotic resistance plasmids in the gut environment (Bakkeren et al. 2019).

Future work will be required to identify the pathogens and clinical infections, where persisters could be targeted to reduce the emergence of new AMR variants.

As these results specifically implicate combination therapy as favouring the development of tolerance, this line of research may lead to a rethinking of therapeutic choices, and may motivate the development of new drugs that are more efficient at eliminating tolerant variants.

3.1.4 Progress in assembling and ensuring accessibility of data

The access to data from different sources relevant to the One Health spectrum is still difficult. The FAIR principles are not commonly applied to research projects and reference centres. Yet combining sequencing data is an essential step to provide a basis for further studies into attribution of the impact of environmental resistomes on AMR in human and animal health.

Prof. Egli coordinated the establishment of a One Health focused sequencing data exchange, the "Swiss Pathogen Surveillance Platform" (www.spsp.ch). The platform was developed together with founding members from the University Hospital Basel (Adrian Egli), University of Basel (Richard Neher), University of Bern (Vincent Perreten) and University Hospital Lausanne (Dominique Blanc, Gilbert Greub). The platform is hosted by the Swiss Institute of Bioinformatics (Aitana Lebrand). The team performed an in-depth requirement analysis integrating diverse stakeholders, e.g., diagnostic laboratories, infectious diseases experts, hospital epidemiologists, cantonal physicians, SwissNOSO, etc. A legal, ethical and governmental framework was established between the institutions. The study was approved by all ethical committees in Switzerland as a multi-centre prospective study (EKNZ lead ethics) and the required legal documents (consortium agreement and data transfer and use agreements) are in place. The team used methicillin-resistant *Staphylococcus aureus* (MRSA) as a proof of concept. By early 2022, about 500 genomes of MRSA genomes had been shared. A controlled vocabulary and the SNOMED CT ontology was used to harmonize the metadata. Bioinformaticians from each institution defined the quality control and analytical pipeline for the sequencing analysis (PMID: 30552858).

Due to the Covid-19 pandemic, the platform was adapted to meet the needs for sequencing exchange of the SARS-CoV-2 surveillance and has shared more than 150 000 SARS-CoV-2 genomes with the FOPH (via an automated interface) and international data repositories such as GISAID and the European nucleotide archive. Prof. Egli invited other NRP 72 researchers to share genomic data with SPSP. SPSP offers to the community a transparent and FAIR research environment to access genome information for research and public health surveillance.

The Swiss Pathogen Surveillance Platform

The Swiss Pathogen Surveillance Platform (SPSP) enables Swiss universities, university hospitals and veterinary medical centres to analyse genetic and epidemiological data on antibiotic-resistant bacteria from many different sources. The SPSP thus provides the basis for monitoring the spread of resistance across humans, animals and the environment in far greater detail and more comprehensively than ever before.

3.1.5 Leveraging the knowledge gained for public health: Conclusions for infection control strategies in healthcare settings aiming to limit transmission of AMR bacteria

For ESBL producers, widespread dissemination in the general population underlines the importance of antibiotic stewardship efforts to limit selection pressure favouring proliferation of antibiotic-resistant strains. However, the data from traveller studies suggest that ESBL strains or resistance plasmids can be readily acquired even in the absence of antibiotic therapies. The impact of infection control measures in healthcare settings on halting further transmission is also likely limited. The finding of a large diversity of strains in clinical, wastewater and food samples suggests that they originate from genetically distinct sources, rather than few sources localized within any of these compartments. We conclude that the current knowledge about the horizontal and vertical spread of ESBL producers is still insufficient to control the problem in Switzerland. New helpful insights might be gained from ongoing studies on phage- or probiotic-based decolonization strategies and research into the principles controlling microbial growth in diverse ecosystems (e.g., NCCR Microbiomes; Gebert Rüf Foundation project "Displacing Multidrug Resistant Bacteria") that are currently ongoing.

3.1.6 The role of the microbiome in facilitating survival and transmission of AMR determinants in complex bacterial communities

In theory, competitors that displace AMR-positive bacterial strains from environmental sites or within hosts could represent tools to fight the spread of AMR and for decolonizing affected patients, but research is in its infancy (Dharmaratne et al. 2021). So far, there is no conclusive data from NRP 72 projects, which could guide any practical applications. However, clinical data and pre-clinical studies using "healthy," antibiotic-susceptible microbiomes in controlling the growth of organisms that can be a health risk, as well as the spread of AMR plasmids and AMR-pathogens within and between hosts, suggest that microbiomes and competitor approaches may work. Results from ongoing studies (NCCR Microbiomes, Gebert Rüf Foundation project) could indeed yield practical approaches and reveal principles that could be harnessed to fight vertical and horizontal spread of AMR. We propose waiting for these results and supporting additional research into this topic to speed up basic discovery and possible practical applications. This could help position Switzerland as a leader in this emerging field.

A paper with contributions of NRP 72 PI Prof. Bonhoeffer, (Tepekule et al. 2019) presents a model to evaluate the impact of treatment history on plasmid-mediated AMR in the gut microbiome. The researchers found that AMR prevalence was best predicted by total days of drug exposure, duration of the drug-free period after last treatment, and the 'centre of mass' of the treatment pattern. This work provides an interesting modelling framework for capturing the role of the microbiome in the selection of AMR and highlights the role of treatment history for the prevalence of resistance. It will be exciting to connect this computational approach with experimental work.

The work of Dr. Bürgmann's laboratory (Ju et al. 2019) on the microbiome and resistome of wastewater treatment plants provides a view of the role of the microbiome outside of the patient or animal system. The researchers showed that in these environmental systems the composition of the microbiome largely determines the associated resistome, with the result that the resistome at the outflow of the treatment plant differs strongly from the inflow. They could further show that the concentrations of some antibiotics in the inflow appear to be correlated with the transcriptional levels of ARG and with the abundance of certain AMR determinants in the discharged wastewater, and further noticed that the relative abundance of many ARGs increased during wastewater treatment. Such results indicated that although the total amount of ARGs declines with such treatment, at least some AMR determinants may experience positive selection during wastewater treatment.

3.1.7 The impact of omics approaches on AMR research

The Swiss Pathogen Surveillance Platform (www.spsp.ch), developed by the NRP 72 project "Development of a Swiss surveillance database for molecular epidemiology of multi-drug resistant pathogens", led by Prof. Egli and with participating centres across Switzerland, represents a big step in this direction. The platform is already set up to link genomics safely and ethically with clinical data. The platform is continuously expanding and discussions to link to the phenotypic resistance data on ANRESIS have started. In future, WGS data from non-clinical sources, e.g., food and environment, could be integrated and this can provide a basis for a far better understanding of the origins and epidemiology of AMR pathogens.

In addition to WGS, NRP 72 has also demonstrated the usefulness of metagenomic sequencing (i.e. sequencing of microbial communities). Metagenomics, coupled with bioinformatics identifying ARG in the data, provides a broad view on the resistance potential in complex microbial communities, such as gut, wastewater, or environmental microbiomes. While the approach is currently expensive and requires high levels of expertise, the rapidly sinking trajectory of sequencing costs and improvements in analysis software as well as computing power indicate that this method will find applications beyond research in the future. The ability to reconstruct draft genomes from such data provides genetic context similar to WGS, and indeed such data can be linked. The SPSP therefore also intends to incorporate metagenomic data. Given the dense network of interactions between a given bacterium, the other microbes and the host (where applicable), we need to evaluate options to also assess these important parameters. This could in principle be achieved by DNA sequence-based profiling, e.g., via 16S ribosomal RNA community sequencing or preferably via metagenome sequencing. Such data could hold key correlative evidence for identifying suitable competitor strains (probiotics) or community features which fuel or prevent horizontal or vertical AMR pathogen spread. The value of such approaches is suggested by preclinical data from mouse colonization and AMR HGT experiments (Wotzka et al. 2019).

3.2 Consolidation of monitoring approaches in a One Health approach

3.2.1 Sequencing as a unifying approach for monitoring across One Health sectors

As described above, a considerable number of projects in NRP 72 relied on WGS or (shotgun) metagenomics to characterize resistant isolates, or ARGs in microbial communities. While the standardization of these approaches presents its own challenges, the sequencing approach has the advantage of providing very rich data that can easily be related across many different sources from the entire One Health spectrum. The metagenomic approach, typically applied to microbial communities from environmental samples, can be linked to WGS via different approaches, ranging from simple similarity searches of reads or assembled contigs to metagenome-assembled genomes (MAGs), draft genomes reconstructed from environmental metagenomes that can be analyzed in similar ways to isolate genomes. Currently, however, this latter approach results in lower quality standards and some caveats regarding their validity. Yuan et al. (Yuan et al. 2021) applied this approach to WWTP metagenomes combined with metatranscriptomics to provide genome-level taxonomy and analyses of actively transcribed ARG to provide a high-resolution qualitative and quantitative analyses of bacterial hosts of ARGs (including information on chromosomally-encoded multi-resistance, pathogenicity, activity, and niche preferences).

Nevertheless, the sequencing approach provides an exciting opportunity to link AMR research across disciplines, supported by the powerful tools provided by modern bioinformatics and open databases. However, taking advantage of this potential will require, crucially, well curated and metadata-rich databases open to contributors from all hospital, veterinary, public health and environmental institutions and researchers.

3.2.2 Facilitating data exchange across One Health sectors

The NRP 72-funded “Swiss Pathogen Surveillance Platform” (SPSP) aimed to address previously mentioned challenges. The SPSP consortium achieved a cross-institutional data transfer and share agreement, which can be easily expanded towards new partners. In addition, we have a successful ethical evaluation of a multi-centre retrospective and prospective research platform, which allows us to monitor the transmission of a large series of public health relevant pathogens. This is the first time in Switzerland that such a contract has existed. Over the past four years, the SPSP team has constructed a data infrastructure that can receive, store, analyze, and visualize high-resolution genomic data from viruses, bacteria, and fungi. It is strongly recommended that the SPSP, like ANRESIS, should become an institutionalized long-term data repository and that all centres involved in WGS of antibiotic-resistant isolates should be encouraged and incentivized to participate. Long-term funding for sustainable development from important public stakeholders should be granted.

Validation of data from Swiss microbiology laboratories to confirm emergence of resistance

Beside phenotypic surveillance, genotypic surveillance of pathogens is also required. The benefits of a genomic surveillance system are currently showcased by the Swiss Pathogen Surveillance Platform (www.spsp.ch), which allowed the Federal Office of Public Health to access SARS-CoV-2 genomes from multiple sequencing institutions and to crosslink sequencing data with reporting forms on an individual case-by-case basis. Without SPSP there would be no data exchange of new circulating variants. In addition to the current focus on human pathogens, the One Health concept needs to be integrated, i.e. data for veterinary and relevant environmental and commensal isolates. A platform to integrate genomic, epidemiological and additional metadata in a shared database would be of greatest value regarding AMR surveillance. This could allow monitoring of the spread of individual AMR genes, plasmids, or pathogens through diverse compartments. These data and strain repositories will also provide an important resource for basic microbiology laboratories to discover the biological principles, which might be harnessed to reduce the spread of AMR (vertically, horizontally).

3.2.3 New One Health control points (monitoring)

While publications of further results deriving from the NRP 72 projects are pending at the time of writing this report, the cumulative knowledge gained by the analyses currently completed supports the following long-term implications to combat further spread of antibiotic resistance:

- Monitoring AMR in wastewater (i.e. wastewater-based epidemiology) may contribute to the surveillance of AMR. In Switzerland, sewage is collected nearly universally and channeled to wastewater treatment plants. This makes WWTP highly interesting as points for monitoring the spread of resistant pathogens, resistance genes or MGEs (e.g., multiresistance plasmids). The usefulness of wastewater-based epidemiology has recently been demonstrated in the Covid-19 pandemic in Switzerland (link). Work done in connection with NRP 72 by Helmut Bürgmann and Sarah Tschudin Sutter has demonstrated the methodology to either isolate organisms of concern or detect resistance genes in sewage or wastewater samples. These data would also allow monitoring of the overall success of measures and interventions in a regionally resolved fashion. Generated data should be integrated into SPSP.

- Food represents another unused control point. Systematic AMR monitoring systems for food are largely missing and should be improved, especially for food items consumed raw.

  Various projects within and outside of NRP 72 have shown that AMR contamination of food can vary but is clearly one route that links directly back to the human gut microbiome and therefore requires special attention. Since Switzerland imports a wide range of foodstuffs, this is also a route by which new AMR determinants can enter the country (Zurfluh et al. 2015). Moreover, the presence of AMR in food typically eaten cooked is not always unproblematic; studies on *Campylobacter* have shown that cross-contamination frequently happens at the consumer (i.e. through insufficient kitchen hygiene) (Santos-Ferreira et al. 2021; Cardoso et al. 2021). Data generated in this area should be integrated into SPSP medicine.

- Pets and companion animals and the small animal clinics that treat them are another control point implicated by NRP 72 research, as described above. Data generated in this area should be integrated into SPSP.

- Continuous surveillance, including WGS of epidemiologically important resistant strains circulating in humans, animals, and foodstuffs is needed to monitor spread, transmission pathways, and sources of antimicrobial resistance.

  NRP 72 has shown the usefulness of the sequencing approach across all sectors of the One Health approach. All data should be collected in a single database like the SPSP developed by the project led by Prof. Egli.

3.2.4 New options for interrupting AMR transfer from the One Health perspective (intervention)

Research conducted in NRP 72 has shown that release of wastewater (usually treated) impacts the resistome of receiving rivers. This impacts not only the water itself, but resistance genes are also detectable in various compartments of the river ecosystem, such as biofilm and sediment. Results obtained within NRP 72 suggest that organisms of concern, such as ESBL or carbapenemase producers, are also released to and detectable in the environment (Bleichenbacher et al. 2020; Zurfluh et al. 2013). Water quality downstream of conventional WWTP may thus be compromised in terms of AMR content. Quality standards and risk-based monitoring of such water for recreational and irrigation use would therefore represent possible interventions. Where direct use of reclaimed water is considered (which, with climate change, may become more relevant in Switzerland), such quality standards should obviously likewise apply.

As NRP 72 research showed that AMR levels can be highly variable in time, and contamination peaks can occur, e.g., through wastewater bypass (combined sewer overflows), additional measures for such situations could be useful. In this respect, measures to decrease the number and volume of bypass events would also represent a useful measure.

Published and ongoing work at the Bürgmann lab indicates that technologies that can considerably mitigate the release of AMR from WWTP are already available in the form of technologies being used to reduce micropollutants. Ozonation at concentrations used in micropollutant oxidation treatment is effective in reducing AMR by two orders of magnitude (Czekalski et al. 2016). Biological post-treatment may lead to a recolonization of treated water with resistant organisms, but ongoing research (Bürgmann, unpublished data) indicates that this phenomenon depends on the filters used for the biological step and can probably be controlled. Results from an activated carbon/ultrafiltration pilot plant showed that membrane filtration is highly effective at removing bacteria from WWTP effluent, and thus also eliminates AMR determinants (Bürgmann, unpublished data).

Work by the groups of Prof. Sidler and Prof. Hummerjohann has indicated that manure is a likely source of AMR transferred to crops. In future, Prof. Sidler intends to study different options of manure processing for their potential to reduce manure AMR content prior to application. This would allow disruption of a potentially important route of AMR transfer to food and thus to consumers.

The role of pets and small animal clinics for the dissemination of AMR was highlighted by NRP 72. While small animal clinics already implement their own hygiene protocols, these new findings should provide motivation to re-evaluate and improve these measures to limit the spread of MDRO between animals and zoonotic transfers from animal to humans.

In food production, new process hygiene criteria and certain practical measures (e.g., decontamination of carcasses) could be considered or re-evaluated in light of new evidence.

3.3 Outlook: Pathways for science and implementation

The systematic surveillance and storage of sequencing and metagenomic data on AMR genes, plasmids and AMR-pathogens should be complemented with rich environmental, therapeutic and epidemiological information. Given the important impact of microbial community composition on the growth capacity of a given bacterial strain and the chances for acquiring AMR, it seems promising to extend surveillance to information about the composition of the microbial community at the sampling site or the microbiome of the respective patient. This could help transform the field from AMR monitoring (as done today) to allow targeted interventions to remove particular ARG, AMR plasmids or strains from a given source or to prevent their transmission and growth at other sites (or in patients).

Considerable challenges remain regarding attribution, i.e. linking AMR reservoirs in, e.g., the population, in domesticated, companion and wild animals and in the aquatic and terrestrial biosphere with cases of resistant infections in human and veterinary medical practice. For these diverse areas, a standardized ontology should be used, introducing a controlled vocabulary. In addition, even more challenging questions arise regarding the impact of these environmental reservoirs and our human impact on them through contamination with potentially selective contaminants or the massive release of bacteria preselected for presence of AMR on MGEs.

4
Recommendations for action

Chapter summary

The NRP 72 Working Group on “Routes and reservoirs of AMR-determinants & One Health AMR surveillance” synthesized the research done in NRP 72 and JPIAMR projects. In close exchange with PIs and stakeholders, it outlined a series of science-based recommendations. These focus on measures that can limit the spread or emergence of AMR from a One Health perspective, as well as on more effective monitoring of AMR across the One Health spectrum.

Four key recommendations are presented:

Key Recommendation 1:
Whole genome sequencing (WGS) should be implemented as a standard surveillance tool.

Key Recommendation 2:
Screening and information campaigns for groups of people with increased likelihood of spreading AMR should be expanded.

Key Recommendation 3:
Monitoring of reservoirs outside the medical settings should be improved.

Key Recommendation 4:
Hygiene and control measures in veterinary clinics, agricultural production and food processing should be improved.

The four key recommendations are each the result of a series of more detailed recommendations that relate to different fields of action. In addition, the synthesis process has produced further recommendations that are not reflected in the key recommendations, such as in the area of research itself. All recommendations are presented in detail in three thematic sections. The first contains recommendations for fully One Health-based surveillance and monitoring. Improved surveillance only makes sense as a basis for improved interventions, which are proposed accordingly in the second section. Finally, NRP 72 also provides a basis for identifying the most urgent remaining research needs. These are briefly presented in the third section.

4.1 How the recommendations were developed

Swiss National Research programmes have the explicit aim of contributing to solving problems. The central goal of this thematic synthesis is therefore to present concrete and practical opportunities for action based on the research conducted in NRP 72. Another objective is to ensure that knowledge and technology transfer is initiated to support the necessary measures. For this reason, the recommendations presented in this chapter were developed in close exchange between researchers as well as with practice stakeholders. First, NRP 72 researchers discussed their own findings during the final presentations of all projects, which took place online from January to June 2021. On this basis, the working group developed a series of recommendations, which they then discussed, honed, and adapted to important practical aspects in several meetings (including online) with the sounding board. In September 2021, these were again presented and discussed at a face-to-face event with a wider circle of practice stakeholders before being finalised in their current form. The recommendations are primarily aimed at new measures, which is why results that merely support existing measures were not taken into account.

Key Recommendation 1

Whole genome sequencing (WGS) should be implemented as a standard surveillance tool.

This includes providing and supporting the “Swiss Pathogen Surveillance Platform” (SPSP) as a crucial infrastructure for sharing whole genome and metagenomics sequencing data and associated metadata with public health relevance. The goal is not to replace the current phenotypic surveillance, but to complement and upgrade Swiss surveillance efforts by creating an interlinked infrastructure with full FAIR access to phenotypic, sequencing and meta-data, and a connected strain repository that should also be linked to current and future international efforts.

Crucially, these measures provide the best solution for creating a basis for detailed high-resolution tracking of the spread of resistance (molecular epidemiology) across all aspects of One Health, enabling targeted interventions and monitoring the effects of pathogen transmission. They also complement the phenotypic characterization of resistant organisms with precise genomic information that can be used to improve therapeutic interventions.

Implementation of this goal requires the creation of a regulatory framework that supports the routine diagnostic use of sequencing technology. It will require investment in maintenance and further development of the platform, e.g., integration of the analysis of new pathogens and visualization of certain data outputs, providing widespread access to sequencing technology to all stakeholders, laboratories and reference centres involved in the AMR surveillance efforts across the One Health spectrum. Initial and long-term investment in creating and maintaining the networked data storage, access and analysis capacity will be required.

Key Recommendation 2

Screening and information campaigns for groups of people with increased likelihood of spreading AMR should be expanded.

This includes:

a. Screening of patients in health-care settings that have recently arrived from countries with a high prevalence of MDR pathogens. Consider information campaigns/strategies for people seeking travel recommendations regarding acquisition of MDROs aiming to inform people about the potential risk and therefore advising to communicate their travel history when they seek medical advice for suspected infection. Consider providing opportunities and counselling for voluntary screening when returning from travel (e.g., at airport clinics)

b. Considering surveillance and screening of patients in long-term care institutions to determine local epidemiology and outbreaks. Screening information will, in addition, guide physicians in choosing empiric treatment when infections are suspected/diagnosed.

c. Informing and advising discharged patients known to be carriers of resistant pathogens on measures to prevent further spread, e.g., in the household care settings.

These measures would fill important gaps in monitoring the introduction and onward spread of MDR bacterial pathogens outside the medical sector and allow targeted interventions (e.g., hygiene measures, isolation).

Key Recommendation 3

Monitoring of reservoirs outside the medical settings should be improved.

We recommend expanding the monitoring of AMR in veterinary clinics (including small animal clinics). Reporting of antibiotic resistances of concern (e.g., production of carbapenemases) should be mandatory. The data from this sector should be integrated into the phenotypic and WGS-based surveillance recommended above.

Similarly, a risk-based expansion of regular monitoring of more food matrices and food imports should be considered. Based on such monitoring, improved hygiene and intervention options should be continuously developed. Surveillance data from important food categories (e.g., ready-to-eat food) should be collected and integrated into surveillance databases.

We recommend establishing wastewater-based surveillance of AMR. While hotspot oriented surveillance (e.g., at airports, hospitals or long-term care facilities) can directly support efforts outlined in recommendation 2, regular population oriented monitoring of antibiotic resistance at wastewater treatment plants (e.g., of larger Swiss cities) and analyses of the overall resistance profile of wastewater in the influents (wastewater-based epidemiology) and effluents (release monitoring) provides important data for the assessment of public-health that is integrative and independent of clinical case reports.

These measures complement existing surveillance and fill gaps in the One Health concept. Comprehensive monitoring allows rapid and targeted interventions to avert hazards and improves the basis for identifying pathways of spread. This opens opportunities for targeted interventions, such as disinfection treatments of slurry or wastewater (e.g., through quaternary wastewater treatment stages).

Key Recommendation 4

Hygiene and control measures in veterinary clinics, agricultural production and food processing should be improved.

Clinics have invested heavily to improve hygiene and antibiotic stewardship in recent years, and other areas of One Health should follow suit.

In veterinary clinics, especially in small animal / companion animal clinics, existing hygiene measures, control measures and antibiotic stewardship should be improved. Appropriate measures prevent the spread of resistant pathogens in this setting and protect staff, owners and the pets themselves.

In agriculture, existing best practice approaches to limit manuring in the production of freshly consumed produce should be strengthened, manure treatments for greater microbiological safety developed, and the quality of irrigation water and other sources of potential contamination with AMR should be taken into account.

The screenings for risk assessment and regular surveillance of AMR in ready-to-eat vegetables, as proposed in Recommendation 2, should be implemented as a first step. The high standards in Swiss production in terms of low antibiotic use should become a quality feature of Swiss animal food production (e.g., labelled products).

Recommendations for One Health surveillance and monitoring

Current surveillance and monitoring approaches need to be improved to fully support the One Health approach to combat AMR, and to take advantage of current technological advances.

Expanding, interlinking and modernizing AMR surveillance in Switzerland

- NRP 72 has clearly demonstrated the advantages of WGS-based surveillance of AMR. It is highly recommended that WGS-based surveillance is established as a standard surveillance tool for MDR bacteria, and that access to the technology and funding is provided broadly to healthcare providers but is also applied in veterinary and environmental monitoring.

- NRP 72 has shown that public, accessible and context-rich WGS data are essential tools to track emergence, spread and evolution of AMR and to combat it effectively. With the Swiss Pathogen Surveillance Platform (SPSP), NRP 72 has developed a platform for efficient, secure and expandable WGS data sharing. It is highly recommended that the SPSP, like ANRESIS, becomes an institutionalized long-term data repository, and that all centres involved in generating WGS data of resistant isolates should be encouraged and incentivized to participate. Reference laboratories generating WGS data focusing on MDR pathogens should share data through SPSP promptly and automatically.

  NRP 72 has also demonstrated the high information value of metagenomic analyses of the resistome in various environments (wastewater, surface water, gut microbiomes, food, soil). Therefore, metagenomic sequence data could also become an important means of identifying strategies to control the spread of AMR and should be included in AMR surveillance databases.

- It should be ensured that WGS-based monitoring is carried out according to internationally harmonised standards. SPSP provides highly interoperable anonymized data which should be shared with international data repositories such as the European Nucleotide Archive and the currently developed EFSA database focusing on food-borne infections. Comparability and access to international databases also allow for source tracking to outside sources.

Establishing new control points for One Health AMR surveillance

- There is strong evidence from NRP 72 and current literature that travellers returning from high-prevalence countries (e.g., on the Indian subcontinent) import MDR bacteria. For this reason, it would be beneficial to develop strategies to monitor this importation (e.g., screening at the intestinal level) and to develop interventions (decolonization strategies). Information campaigns and opportunities for voluntary screening should be considered to obtain a better picture of the extent and risks of MDRO introduced by travellers. If outbreaks of high risk MDRO occur, strategies to implement mandatory screening should be considered

- We further suggest that patients admitted to health care facilities who have travelled to high-risk regions in the last 3–6 months undergo mandatory screening for MDR bacteria prior to hospital admission, even if they do not present additional risk factors. An agency, e.g., the Federal Office of Public Health, should be tasked with creating and maintaining a list of high-risk regions. MDR

bacteria detected during screening – especially those belonging to the Enterobacterales – must be characterized phenotypically, and genetically using WGS. This information is essential to prevent the importation of new and life-threatening MDR bacteria that can later spread to the community and hospital settings.

- The authors of this thematic synthesis are aware that the proposed measures are not easy to implement, and that successful decolonization strategies have yet to be developed. However, as it becomes increasingly clear that the importance of this import pathway outweighs most domestic transmission routes, we want to initiate further reflection and activities on this subject.

- NRP 72 has identified several environmental control points that can provide useful information on AMR dissemination for One Health-oriented AMR surveillance. Wastewater monitoring for AMR (i.e. wastewater-based epidemiology applied to AMR) should be further developed and established as a routine monitoring tool that allows for tracking of the spread of AMR on a population basis. Monitoring of airport sewage (Heß et al. 2019) could support the monitoring of import by travel, while monitoring of communal wastewater can support surveillance of AMR spreading in the population and outbreak tracking (Blaak et al. 2021). A combination of indicator-based monitoring (i.e. for ARG and MDR bacteria and general indicators of AMR abundance) and metagenomic sequencing-based resistome surveillance would provide powerful tools for early detection of AMR spread and outbreak monitoring. The research community has converged on promising candidates for monitoring AMR in environmental samples over the last decade. A political effort is now needed to specify and then implement standard parameters, procedures, and safe limits for environmental resistance monitoring. Ideally, these should be internationally harmonized.

- Additional food items should be considered for AMR monitoring following a systematic risk analysis. Foods consumed raw and manured or irrigated during production, foods known to be a source of household cross-contamination, and imported food, especially from regions at increased risk of emerging AMR (e.g., South Asia and North Africa) should be given special consideration in monitoring.

- NRP 72 research showed that small animal clinics can be involved in AMR outbreaks. They should be included in AMR surveillance. In the context of NRP 72 research projects, the role/importance of small animal hospitals/clinics in spreading AMR (e.g., carbapenemase-producing Enterobacterales) was demonstrated. An obligation to implement hygiene concepts could counteract this. Of course, such obligations only make sense as part of a harmonized approach across all aspects of veterinary care and human healthcare. In addition, a recently published study showed that raw meat-based diets for dogs and cats are an important way of spreading ESBL-producing Enterobacterales at the food chain / pet interface. AMR monitoring and surveillance for pets or small animal clinics in Switzerland is now becoming available. It appears reasonable that such data should be fully integrated into databases covering the One Health concept.

The proposed One Health surveillance and monitoring efforts (see above) will not only provide an improved overview of the resistance situation in Switzerland in full appreciation of the One Health concept, but also provide the basis for future modelling efforts and attributions studies that link AMR across reservoirs with the actual infection risks of humans and animals.

Recommendations for interventions

Information sharing and practical advice for stakeholders

Knowledge gained in NRP 72 on AMR evolution, ARG transfer and environmental AMR reservoirs must be made available to stakeholders and, where possible, recommendations made for practical consequences. Suitable information products must be developed jointly by researchers and stakeholders.

- Antibiotic tolerance has been shown to be a relevant mechanism in the evolution of AMR. It is recommended that the medical community is made aware of this mechanism, and in particular of the resulting detrimental role that combination therapy can play in chronic infections. Further actions might be warranted as additional data come in (e.g., from clinical studies, NCCR Antiresist).

- NRP 72 research has identified the transmission of MDR bacteria from patients discharged from hospital to the community/households, especially to caregivers, as a possible route of AMR dissemination. We recommend that awareness campaigns are initiated, and that discharged patients are informed about being carriers of AMR. Caregivers of such patients should receive instructions on minimizing their risk of acquiring and spreading AMR. Similar strategies could be followed for travellers identified as carriers of MDR bacteria.

- NRP 72 research provided several recommendations for animal production. For instance the observation of AMR spread between animals of a herd, and considerable persistence of AMR even without the use of antibiotics suggests that controlling such spread is important, favouring measures like all-in-all-out models in pig fattening.

Recommendations for new One Health intervention options

- NRP 72 provided new evidence that AMR is discharged into the environment with wastewater, that potentially problematic MDR bacteria are present in the environment as a consequence, and that they persist in the environment over considerable distances. According to the precautionary principle, it is recommended to reduce such inputs to the extent possible within the constraints of current operations of wastewater management. Upgrading of WWTP with micropollutant-removal stages (e.g., ozonation, UV treatment, membrane filtration, granulated activated carbon) is currently in planning or already underway in Switzerland. This presents an opportunity to ensure their optimal operation for the additional purpose of reducing the load of resistant bacteria. Mixed sewer overflows were shown to be an important source, and an emphasis should be put on reducing these inputs in the course of projects for the renewal or expansion of the Swiss wastewater management systems. The discharge of untreated sewage should be minimized.

- Process hygiene criteria should be established for specific food categories following a systematic assessment of consumer exposure to AMR from food (https://www.frontiersin.org/articles/10.3389/fmicb.2018.00362/full).

- In meat production, decontamination of carcasses and the respective necessary adaptation of the legal basis of the respective food law regulations should be considered in light of current evidence. “Antibiotic stewardship” labels for consumer information could provide incentives that favour livestock production with low antibiotic use and best practice in terms of reducing AMR.

Recommendations for future research

NRP 72 has provided solid foundations for action in many areas, as outlined above. In some other areas, further research efforts could provide additional tools in the struggle against AMR in the future.

- NRP 72 has produced a wealth of data across the One Health landscape, including humans, animals, the food chain, and the environment as reservoirs of AMR. However, a dedicated quantitative attribution study linking various AMR reservoirs as potential sources of AMR problems in the human clinical context has not been attempted within NRP 72. This would require a combination of dedicated and harmonized prevalence studies, standardized molecular methods (WGS), and modelling of exposure through various transmission routes. Only such studies can provide answers to the central question of evaluating risks associated with the various One Health sectors. This would be of great value to prioritize interventions and monitoring targets. It is recommended to launch such a research and synthesis effort. Support for SPSP and further research projects investigating AMR prevalence in various environments will sustain this essential effort.

- Several projects have found initial evidence of possible innovative interventions that may reduce the spread of AMR, e.g., in the human or animal gut (glycerol in chicken feed). It is recommended that such interventions are further explored to determine their potential as a complementary treatment during antibiotic therapy. Research into such interventions and decolonization strategies should be encouraged.

In the opinion of the authors, it is unlikely that the problem of AMR can be ultimately “solved”. AMR is the result of evolutionary processes that we can influence, and there is much we can do to increase the odds of more favourable outcomes. But “life will find a way” (Michael Crichton, “Jurassic Park”). Therefore, research into new problems that will arise, constant surveillance, re-evaluation of old strategies and development of new ones with the best means that science can provide will continue to be necessary for a long time to come. Maintaining a strong AMR research landscape in Switzerland is an investment in the future.

NRP 72 and JPIAMR projects contributing to the thematic synthesis

Contribution of natural transformation to the transmission of resistance genes in hospital-acquired pathogens
https://data.snf.ch/grants/grant/167061
Project lead: Melanie Blokesch | EPF Lausanne

Towards quantification of the contribution of plasmids to the spread of antibiotic resistance
https://data.snf.ch/grants/grant/167121
Project lead: Sebastian Bonhoeffer | ETH Zurich

Swiss River Resistome – identity, fate, and exposure
https://data.snf.ch/grants/grant/167116
Project lead: Helmut Bürgmann | EAWAG

Development of a Swiss surveillance database for molecular epidemiology of multi-drug resistant pathogens
https://data.snf.ch/grants/grant/177504
Project lead: Adrian Egli | University of Basel

Whole Genome and Plasmid Sequencing for MDR Enterobacteriaceae Simultaneously Isolated from Multiple Human and Non-Human Settings: Deciphering Impact, Risks, and Dynamics for Resistance Transmission and Spread
https://data.snf.ch/grants/grant/177378
Project lead: Andrea Endimiani | University of Bern

Insights into the role of phages on the bacterial resistome
https://data.snf.ch/grants/grant/167090
Project lead: Elena Gomez-Sanz | ETH Zurich

Understanding and modelling reservoirs, vehicles and transmission of ESBL-producing Enterobacteriaceae in the community and long term care facilities
https://www.jpiamr.eu/projects/modern/
Project lead: Stephan Jürgen Harbarth | University of Geneva

Resistome in the pig farms: Comparison of the breeding and fattening units with a One Health approach
https://data.snf.ch/grants/grant/177452
Project lead: Markus Hilty | University of Bern

Tracking antibiotic resistance from environmental reservoirs to the food chain
https://data.snf.ch/grants/grant/167068
Project lead: Jörg Hummerjohann | Agroscope

Tolerance as a potential reservoir for the development of antibiotic resistance
https://data.snf.ch/grants/grant/167080
Project lead: Urs Jenal | University of Basel

Modelling the spread of antibiotic resistance genes between chicken and human
https://data.snf.ch/grants/grant/177502
Project lead: Christophe Lacroix | ETH Zurich

Risk of companion animal to human transmission of antimicrobial resistance during different types of animal infection
https://www.jpiamr.eu/projects/pet-risk/
Project lead: Vincent Perreten | University of Bern

Dynamics of transmission of polymyxin resistance genes in Enterobacteriaceae; from the environmental source to the patient
https://data.snf.ch/grants/grant/177381
Project lead: Laurent Poirel | University of Fribourg

Escherichia coli ST131: a model for high-risk transmission dynamics of antimicrobial resistance
https://www.jpiamr.eu/projects/st131ts/
Project lead: Laurent Poirel | University of Fribourg

Partnership against Biofilm-associated Expression, Acquisition and Transmission of AMR
https://www.jpiamr.eu/projects/beat-amr/
Project lead: Qun Ren | EMPA

Intervention of antimicrobial resistance transfer into the food chain
https://www.jpiamr.eu/projects/inart/
Project lead: Xaver Sidler | University of Zurich

Transmission of ESBL-producing Enterobacteriaceae and their mobile genetic elements – identification of sources by whole genome sequencing
https://data.snf.ch/grants/grant/167060
Project lead: Sarah Tschudin Sutter | University of Basel

Piloting on-site interventions for reducing antimicrobial use in livestock farming in emerging economies
https://www.jpiamr.eu/projects/reduce-amu/
Project lead: Thomas Van Boeckel | ETH Zurich

Abbreviations and acronyms

AMR - antimicrobial resistance
ARG - antimicrobial resistance gene
ESBL - extended-spectrum beta-lactamase (producers)
MDR - multi-drug resistance
MDRO - multi-drug resistant organisms
MGE - mobile genetic element
NRP - National Research Programme
SPSP – Swiss Pathogen Surveillance Platform
WGS - whole genome sequencing
WWTP – wastewater treatment plant

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