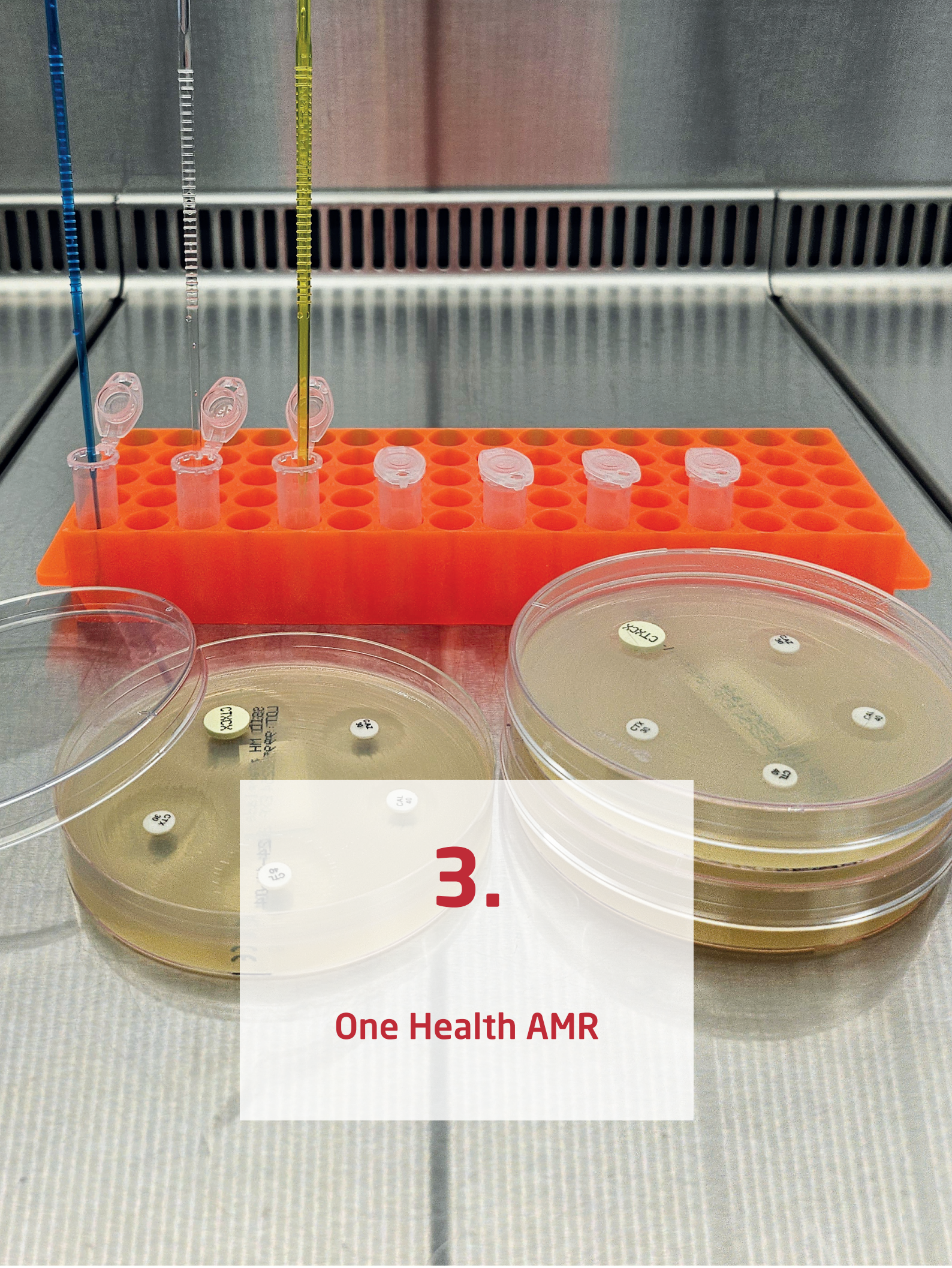


3.

One Health AMR

3. One Health AMR

3.1 Introduction

One Health is defined as a unified approach to optimize the health of people, animals and the environment, under which multiple sectors must collaborate at varying levels [www.who.int].

DANMAP was established with the aim of understanding the drivers of AMR in humans and in the livestock industry and their interconnectedness. The monitoring programme has always been considered an integrated approach to research and surveillance, but with integration taking place at the coordination level rather than at the level of data collection and management. Hence, data are stored in separate databases by the animal and human sectors, however interpretation of results is done in cooperation whenever possible. Moreover, integration happens when discussing resistance findings in indicator and pathogenic bacteria and using it as a basis for recommendations and treatment guidelines. Finally, DANMAP supports the development and definition of strategies and action plans to reduce AMR in a collaborative manner, by monitoring sector specific targets and by fostering the dialogue between different actors and stakeholders across the human and animal sectors.

But antimicrobial resistance is a One Health challenge that extends beyond human and animal populations into the wider environment. Despite its undoubtful integrated nature, the DANMAP programme continues to fall short on the monitoring of antimicrobials and antimicrobial resistance in the environment (see DANMAP 2021, Chapter 3, Textbox 3.1). Environmental compartments such as surface waters, wastewater systems, soils, sediments, and wildlife can act as reservoirs, mixing points, and transmission pathways for antimicrobial-resistant bacteria, resistance genes, and antimicrobial residues. Consequently, there is growing international interest in establishing surveillance systems capable of detecting AMR emergence, tracking dissemination pathways, and assessing environmental drivers of resistance selection.

Despite the growing focus in this One Health pillar, environmental AMR monitoring is still at a relatively early stage of development, not only in Denmark, but worldwide. Key challenges include identifying suitable indicators, selecting representative sampling locations and matrices, harmonizing analytical methods, and determining how environmental data can inform risk assessments and policy decisions (see Textbox 3.1).

3.2 Integrated reporting

An aim of the DANMAP program has always been the in depth understanding of the possible relationship between the animal, food and human sectors concerning antimicrobial usage (AMU) and development of antimicrobial resistance (AMR). In addtion to the AMU and AMR data presented in the other DANMAP chapters, investigations of the interplay between sectors imply knowledge about antimicrobial resistance mechanisms, routes of transmission of pathogens and factors that may enhance the impact of resistance transmission.

This again calls for further harmonised data collection, to define common denominators and units and to be able to perform cross-analyses on data from both the veterinary and human sectors.

At the EU level, an attempt to perform cross-analysis has been made since 2015 in the JIACRA reports [JIACRA IV, 2019-2021, ECDC, EFSA, EMA; 2024]. At the national level, even in a country such as Denmark with a long-established detailed monitoring system based on stable delivery of high-quality data, there are a few challenges in the implementation of integrated data analysis. Data are collected in the animal, food and human sectors often under different premises - following different legislation and purposes, with varying sampling strategies and magnitudes. Some of these obstacles can be circumvented by investigating the genetic characteristics of resistant bacteria recovered from livestock animals, meat and humans.

Here, we map the frequency of multi-locus sequence types (MLST) and resistance genes and mutations of extended spectrum beta-lactamase-producing *E. coli* (here abbreviated to ESBL Ec) recovered from livestock animals and meat and from humans with bloodstream infections. Several recent studies [Aziz, et al 2024. Microbiology Spectrum. 12. e0341523; Nadimpalli et al 2023, Frontiers in Ecology and the Environment. 21. 10.1002/ fee.2639; Liu et al 2023. One Health, 16: 100518; Roer, et al. 2019. J Antimicrob Chemother 74(3):557-560; Valcek, et al. 2019. J Antimicrob Chemother 74(8):2171-2175] report possible zoonotic transmission of ESBL Ec, both in high-income and low- and middle-income countries, underlining the importance of monitoring the occurrence of these bacteria in animals and humans, and assessing the possibility of transfer across sectors. A recent modelling study [Brinch et al. 2025. Zoonotic Diseases 5(1): 7] performed in Denmark including the ESBL Ec isolates that have also been reported in DANMAP, has contributed to the body of work that assesses the likelihood of zoonotic transmission of ESBL Ec (see DANMAP 2024, Textbox 3.1).

ESBL/AmpC-procuding *E. coli* is one of the organisms that DANMAP has monitored by use of WGS of isolates from animals and imported and domestic food products (see chapter 7, section 7.3.1), as well as from human infections (see chapter 8, section 8.3.1). In this chapter, we investigate possible relationships between ESBL Ec from different sources in Denmark.

3.2.1 Genotypic comparison of ESBL/AmpC-producing *E. coli* from humans, animals and food

Since 2022 (DANMAP 2021, Chapter 3), DANMAP has compared the distributions of multi-locus sequence types (MLSTs or STs) and ESBL/AmpC genes and mutations among ESBL Ec from humans, food-producing animals and meat to identify any major overlaps between sectors suggesting a zoonotic link.

In the present report, we added new data from 2025; 120 isolates of animal origin and 188 isolates from humans, totaling a dataset of 2,579 ESBL isolates from humans and animals from 2018 through 2025. The 1,639 human isolates were clinical isolates from bloodstream infections sent voluntarily from the departments of clinical microbiology to the SSI reference laboratory for antimicrobial susceptibility testing. The animal and meat isolates (broilers: 110, broiler meat: 164, cattle: 75, beef: 53, pigs: 329, pork: 67, and turkey meat: 142) stem from the EU mandatory screening programme from healthy animals and meat products (see Chapter 10 for more information).

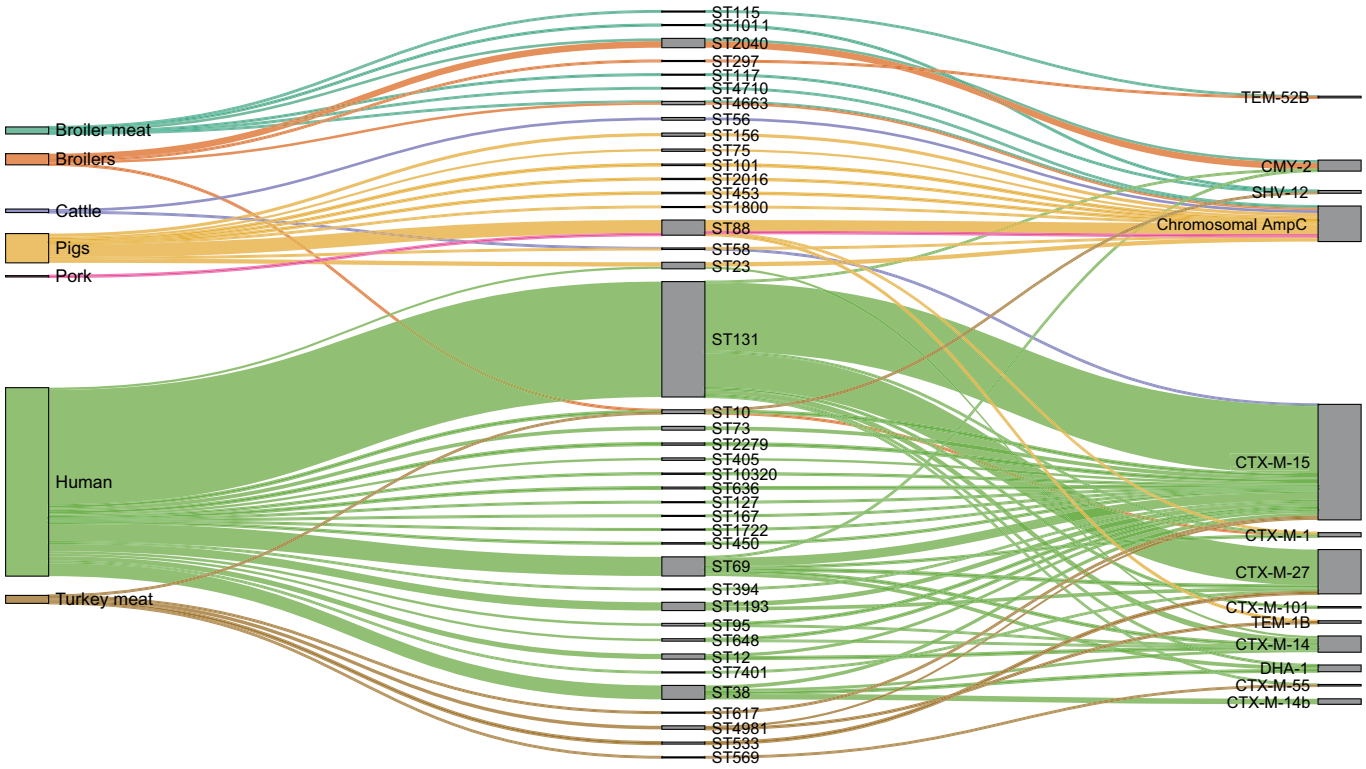
Each isolate has been sequenced as part of the surveillance activities, and the MLST and ESBL/AmpC genotype were extracted from the whole genome sequence. For an overview of sequence types and ESBL/AmpC genotypes detected in 2024 in *E. coli* from bloodstream infections, and from pigs, cattle, pork and beef, see Chapter 8 (Tables 8.14-16), and Chapter 7 (Table 7.3), respectively. For the purpose of the visual demonstration of the abundance of STs and resistance genes in the different reservoirs, we selected only flows of five or more isolates, thus limiting the analysis to a selection of data (Figure 3.1). The results described below refer to the selected isolates.

As in the previous years, limited overlap was found in both STs and ESBL/AmpC genes and mutations in isolates from humans vs. animals and food (Figure 3.1).

Regarding the distribution of MLSTs, one or few sequence types predominated among the isolates of each source. Isolates from humans were mostly from ST131, followed by ST69 and ST38 (see also Chapter 8, Table 8.16). The most abundant STs of animal and meat isolates were ST2040 for broilers and broiler meat, ST4981 for turkey meat, ST88 for pigs and pork, and ST56 for cattle. In accordance with former findings (see DANMAP 2015, Textbox 7.3), ST23 was found in both humans and pigs, although the ESBL/AmpC genotype differed between the human and pig strains. The pig isolates from ST23 harboured AmpC C-42T mutations, whereas the human isolates harboured the ESBL gene CTX-M-14. Additionally, ST10 was found in broilers, turkey meat and humans, but also harbouring different resistance mechanisms – CTX-M-1, SHV-12 and CTX M-15, respectively.

The AmpC plasmid-mediated gene CMY-2, and the ESBL genes CTX-M-1, CTX-M-15, CTX-M-27 were found in both humans and food-producing animals or meat. Additionally, also the ESBL genes CTX-M-55 and SHV-12 were detected across sectors. Interestingly, turkey meat isolates were those with the largest overlap with human isolates regarding the detected ESBL genotypes, including carriage of CTX-M-15 and CTX-M-27 (the two most common ESBL genes among human isolates; see also Chapter 8, Table 8.15), as well as CTX-M-55, although by different MLSTs. The CMY-2 AmpC gene was almost exclusively found among isolates from broilers and broiler meat from ST2040, but also in human isolates of ST69 and ST131. The CTX-M-1 gene was mostly found among human isolates, but also in pig isolates of ST88 and ST10 isolates from broilers. All isolates from cattle harboured a C-42T mutation, which was not detected among the selected human isolates.

Figure 3.1 A Sankey diagram comprised of 1,624 ESBL Ec MLST-gene/mutation combinations from humans, animals and food showing the relationship between the isolates' source, sequence type and ESBL/AmpC gene or mutation DANMAP 2025



In general, sequence types seem to strongly associate with species, whereas there is more variance in combinations of STs and ESBL/AmpC genes and mutations. In the 2018 DANMAP report Textbox 7.2, Roer, et al., used whole genome sequencing on a similar, but smaller, dataset to investigate for possible zoonotic links. A possible link was found in ST69/CTX-M-1, but the SNP-distance was not indicative of an outbreak or a direct transmission, but rather of a clonal relationship.

In conclusion, it remains challenging to find clear evidence of zoonotic transmission of ESBL Ec between animals and humans in Denmark, within the investigated time frame of eight years, and when considering the occurrence of ESBL/AmpC genotypes in different sequence types. Many genes encoding for ESBL-producing phenotypes can be harboured by plasmids, and hence horizontally transmitted between different, reservoir-specific *E. coli* sequence types. The analysis here performed does not allow to investigate the occurrence of plasmid transmission across reservoirs. Further research into slow transmission over longer time spans, as well as more in-depth genomic analyses are of continued interest and on-going.

3.3 Towards a future of environmental AMR surveillance

There is recent increased international focus on developing practical monitoring approaches and on defining the scientific framework needed to support routine environmental AMR surveillance. Such initiatives occur both within research, as well as national AMR monitoring programmes, and focus on various environmental compartments.

3.3.1 AMR monitoring in wildlife

A study performed in Germany investigated whether wildlife can be used as indicator of AMR circulating in the environment [Plaza-Rodríguez et al. 2021. Front Vet Sci. 7:627821]. Samples were collected from wild boar, roe deer, and wild ducks and geese across Germany through the national zoonoses monitoring programme. While the overall prevalence of resistant bacteria was relatively low, ESBL/AmpC-producing *E. coli* were detected in a small percentage of animals across all sampled species, and resistance to various critically important antimicrobials was sporadically identified. The authors recommended continuous wildlife surveillance as an early-warning system for environmental AMR monitoring.

A more recent systematic review focused exclusively on wild birds and reinforced their potential as large-scale, cost-effective sentinel species for environmental AMR surveillance [Cocos et al 2025. Antibiotics 14(9):905]. This systematic review synthesized evidence from studies investigating antimicrobial-resistant *Enterobacteriaceae* in free-ranging wild birds across Europe and found that resistant bacteria are widely distributed among European wild bird populations, including numerous reports of ESBL-producing isolates across bird species and geographic regions. Furthermore, birds frequenting urban areas, landfills, wastewater-affected environments, and agricultural

landscapes generally exhibited higher prevalence of resistant *Enterobacteriaceae*, supporting evidence of the important effect of anthropogenic AMR pollution.

In addition to wild birds, small mammals can complement bird-based surveillance and provide information on local environmental AMR contamination. Hedgehogs living near urbanized or human-impacted environments have been identified as useful indicators of anthropogenic AMR pollution, also in Denmark [Rasmussen et al 2019. PLOS ONE 14(9): e0222031]. A recent study performed in Italy also investigated the occurrence of β-lactam and carbapenem resistance in hedgehogs [Di Francesco et al 2026. Vet Res Commun 50:267] and identified several β-lactam resistance genes in hedgehog-associated bacteria, as well as genes associated with resistance to critically important antimicrobials, including carbapenems.

These and other similar studies support the idea that AMR surveillance programs should not focus solely on the human sector and livestock production systems but should also include wildlife inhabiting agricultural and urban environments. Wildlife species may acquire resistant bacteria from human- or livestock-associated environmental contamination and subsequently contribute to their further dissemination. A recent review [González-Martín et al 2026. Animals 16(12):1859] examined the role of interactions between wildlife and livestock occupying shared habitats in the dissemination of ESBL/AmpC-producing *E. coli*. After reviewing the available evidence, the authors concluded that bacteria could move in both directions across the wildlife-livestock interface, and that a One Health approach should be adopted when designing AMR surveillance programs.

3.3.2 AMR monitoring in surface water

Parallel initiatives across the world are focusing on developing methods and protocols for AMR monitoring in surface waters.

In the United States, a study conducted within the National Antimicrobial Resistance Monitoring System (NARMS) evaluated advanced genomic methods for AMR surveillance in surface waters [Ottesen et al 2022. PLOS Water 1(12): e0000067]. Sampling was performed both in an urban creek located near a hospital and at a reservoir used for recreation and drinking water supply, and the samples were analysed both with direct shotgun metagenomics and with quasimetagenomics (shotgun sequencing after selective enrichment). This study showed that quasimetagenomics detected NARMS priority AMR targets to a wider extent than standard metagenomics. Furthermore, quasimetagenomics enabled detection of clinically important resistance genes, resistance-associated plasmids and differences in resistance-gene abundance between the two sampled sites.

Similar initiatives to monitor surface waters were also recently taken in Europe. The European Environment Agency (EEA) and partners have launched a pilot study to establish harmonised AMR monitoring in European surface waters [https://water.europa.eu/freshwater/europe-freshwater/amr/documentation]. The study findings have informed subsequent guidance documents that aim at supporting harmonized monitoring and data reporting across Europe. This represents one of the first coordinated efforts in Europe to move environmental AMR surveillance from research into routine regulatory monitoring.

3.3.3 AMR monitoring in wastewater

Wastewater is increasingly viewed as a strategic AMR surveillance matrix because it integrates resistance signals from large human populations and can serve as an indicator of the potential pollution from wastewater plants to the environment.

The revised Urban Wastewater Treatment Directive (EU) 2024/3019 introduced, for the first time, a requirement for monitoring AMR in urban wastewater as part of the EU's One Health response to AMR. The Directive recognizes wastewater as an important pathway through which resistant bacteria and antimicrobial resistance genes originating from human populations enter the environment, and it requires Member States to implement AMR monitoring in wastewater from large urban agglomerations (those serving 100,000 population equivalents or more), develop harmonised surveillance approaches, and generate data. It represents thus a major shift from research-based surveillance towards routine, harmonised monitoring of AMR in wastewater across Europe.

Research focused on AMR detection in wastewater has been ongoing for several years and has contributed with evidence that supports regulatory initiatives such as those of the revised Urban Wastewater Treatment Directive. The latest study published by the Global Sewage Consortium represents one of the largest investigations of AMR in wastewater to date [Martiny et al 2025. Nature Communications 16:10278]. Researchers analysed 1,240 sewage samples from 351 cities across 111 countries to understand the factors shaping the global distribution of antimicrobial resistance genes (ARGs) in urban wastewater. The study applied metagenomic sequencing of sewage samples to characterize the acquired and latent ARGs in the resistome. Findings suggest that wastewater monitoring programmes should focus not only on clinically important acquired resistance genes but also on the broader environmental resistome that may serve as a source of future resistance threats.

3.3.4 Future perspectives

Collectively, these and other similar studies and regulatory initiatives will support the development of future national and international One Health AMR surveillance systems, by providing valuable insights into the strengths, limitations and needs of environmental monitoring. Eventually, such efforts may enable the future implementation of multi-layered frameworks in monitoring programmes like DANMAP, where AMR monitoring in wildlife, surface-water, wastewater and other environmen-

tal compartments is considered. Some countries have already initiated that journey. For example, the study on USA surface waters supports the plans to implement surface water AMR monitoring in the NARMS program. Another example is the One Health Report on Antimicrobial Use and Resistance in Belgium (BELMAP), which has already introduced the reporting of antimicrobial residues in surface water, wastewater, sediments, and agricultural environments, as well as information on fungicide application and fungal resistance, and data on sales of biocidal products such as disinfectants, and antiseptics, which can act as co-selective agents for antimicrobial resistance.

The DANMAP partners, Statens Serum Institut and the National Food Institute, integrate various consortia of international studies that aim both at understanding AMR ecology in the environment and assessing how the future of AMR environmental monitoring can look like. Examples of such consortia are the European Partnership on Animal Health and Welfare [https://www.eupahw.eu] and the EU-WISH project [https://www.eu-wish.eu].

DANMAP is committed to introducing environmental monitoring as the missing One Health pillar in the programme. The 2024 revision of the Urban Waste Water Treatment Directive is expected to foster new national environmental monitoring activities in the near future, which will hopefully strengthen the focus on AMR of the already existing national surveillance program for aquatic environment and nature (NOVANA) (see DANMAP 2024, Textbox 3.2). Along that journey, thorough considerations regarding conceptual frameworks and data requirements must be taken.

Mikkel Lindegaard and Ana Sofia Ribeiro Duarte
For further information: Mikkel Lindegaard, ldd@ssi.dk
or Ana Sofia Ribeiro Duarte, asrd@food.dtu.dk

Textbox 3.1

Environmental AMR surveillance: From detection to decisions

The purpose of environmental AMR surveillance

Taking action against AMR without surveillance data to base mitigation decisions on has been likened to "flying blind" [1]. Along the same lines, one could argue that AMR surveillance without a clear objective is similar to getting into the cockpit without knowing the directions to the destination. It would take an awful lot of time and resources figuring out where to go with the plane in the air, which is why commercial air flights are – of course – planned meticulously. The same should be true for AMR surveillance: we need to know *why* we want to do surveillance and *what* we want to achieve before we start. This is particularly true for environmental surveillance, which easily becomes a task of figuring out what everything AMR-related looks like everywhere, all the time.

In clinical and veterinary AMR surveillance, surveillance data could influence the prescription and use of antimicrobials and can often be specific to particular pathogens and antimicrobial treatment regimes. The value then comes in the form of damage control, but also an opportunity to get ahead of the curve and detect emerging AMR threats before they become common in clinics or among production animals [2].

Such direct application links are harder to establish for environmental AMR surveillance. One could organize the environmental surveillance efforts into three different overarching 'use cases':

- Track AMR circulating in populations (e.g. a city or a farm) through wastewater.
- Evaluate environmental releases, dissemination and potential exposure to resistant microbes.
- Identify emerging resistance mechanisms before they become established clinical or veterinary problems.

None of these use cases does by itself provide a clear actionable outcome for the surveillance. Say that an increase in a gene providing resistance to a last-resort antibiotic is detected in Copenhagen wastewater; what action would that warrant? Covid-style lockdowns? Screening of incoming patients to hospitals? Protective measures towards the elderly? All these options are disproportional to the evidence, making it clear that we need prior definitions of:

1) What risks are we aiming to reduce through AMR surveillance?
2) What meaningful actions could we take based on surveillance to reduce these risks? Who would be responsible for taking these actions?
3) What would be the different 'trigger levels' measured in surveillance to initiate such actions?
4) How can we assess the efficiency of the measures taken?

The risks and actions landscape

The risks associated with AMR can be divided into two major groups: transmission risks and emergence risks, and both can be further subdivided. The transmission risks constitute the spread of resistant pathogens or mobile genetic elements carrying antibiotic resistance genes in various settings (hospital, agriculture, wastewater and so on). Actions to reduce those transmission risks involve hygiene, isolation of carriers, separation of animals, reducing releases of AMR into the environment and measures to avoid exposure of animals and humans to environmentally transmitted AMR [2]. Those measures are well understood, but generally not easy or popular to implement.

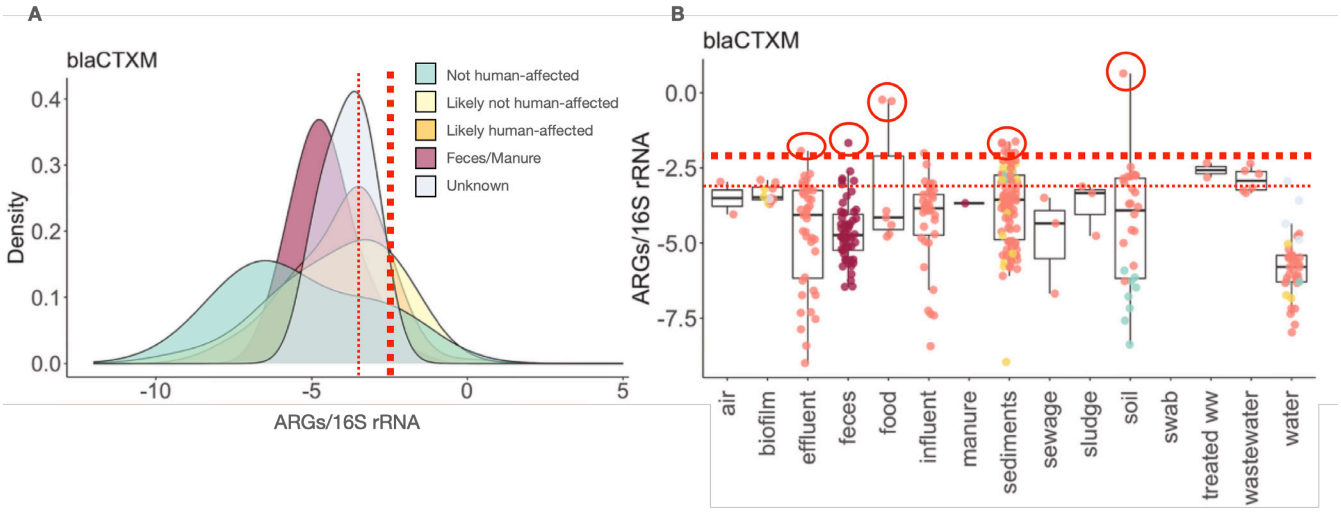
The emergence risks, on the other hand, are connected to new forms of resistance appearing in pathogens, through selection, mutations, or transfer of new resistance genes into pathogens. Here, any mitigation efforts would need to target the flow of AMR into human or animal populations, or trying to contain transmission early. For this type of risks, only two courses of action are available: preventive measures to avoid AMR flow into humans and animals, or early-warning monitoring coupled to isolation of patients or sick animals [3].

continued ... Textbox 3.1

What is a meaningful change?

The other major issue is to couple actions to measurements. What detections would warrant a certain set of responses? Here, it is crucial to define what are the normal, expected variations of a given AMR target in a specific setting. This requires repeated sampling to establish baselines and understand, for example, seasonal variation [4]. It may also be important to consider what the host organism and genetic context is for a particular resistance gene, and if it is associated with mobile genetic elements. Once such background data is obtained, it is possible to make priorities and set levels that would trigger specific actions (Figure 1).

Figure 1 Example of a 'trigger level' method for AMR surveillance DANMAP 2025



Example of the 'trigger level' method suggested in Abramova et al. (2023) [4], using 10 times the third quartile (3Q) abundance values for a given ARG to determine the upper limit for what should be viewed as a deviation from normal background levels in the environment. In the example, the overall levels of blaCTX-M is shown with the 3Q indicated in thin dots and the 10x trigger level indicated by the thick dotted line (A). in (B), the same approach is taken but on an environment basis, showing the samples that would have triggered potential actions in red circles at the top.

Beyond established surveillance targets

For the emergence risks, AMR surveillance is dependent on being able to identify new forms of high-priority resistance before it has appeared (or become widely spread) among pathogens. In order to do that, we need to know what forms of resistance we are looking for. That, in turn, means that there is a need for characterizing and prioritizing new forms of AMR that have a high potential to transfer to pathogens. Recent work has shown that there is a substantial reservoir of resistance genes that conventional analyses overlook [5]. Most of these genes exist in harmless environmental or commensal bacteria and never make it into pathogens; the important task is to identify the ones that are able to early. This would involve investigating candidates for evidence of resistance function, mobility and occurrence in species that are close relatives to pathogens. For such purposes, it is also important to keep track of non-clinical resistance genes across strains of bacteria, environments and studies. This is one of the purposes of the recently launched CLEVER database (https://microbiology.se/clever/), that keeps track of both clinically established and latent resistance genes, giving them names and organizing them by verification status and mobility. This type of information can help prioritizing resistance genes that are likely to become clinical problems, rather than blindly looking for all forms of AMR.

Conclusions

In the end, AMR surveillance shouldn't start with detection and work towards decisions. It should start with the decisions and work its way backwards to the appropriate settings, methods and frequencies for detection. All AMR surveillance efforts, but particularly those in the environment, should be evaluated by whether they identify important changes, inform appropriate responses and have measurable intervention effects. With the EU now strongly pushing for wastewater surveillance of AMR, the issue of purpose has become urgently pertinent [6]. The question we should all ask before we venture into surveillance should be: "What decision will this measurement enable?"

Johan Bengtsson Palme
For more information: johan.bengtsson.palme@chalmers.se

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