One Health AMR – new perspectives

Duarte, Ana Sofia Ribeiro; Lindegaard, Mikkel; Muller-Pebody, Berit; Sönksen, Ute Wolff

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ONE HEALTH AMR – NEW PERSPECTIVES

3. One Health AMR – new perspectives

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.

DANMAP has always been considered an integrated research and surveillance programme, but with integration taking place at the decision-making and implementation level rather than at the level of data management. Hence, data are stored in separate databases by the animal- and human sectors, although interpretation of results is done in cooperation. Moreover, integration happens when deciding which common indicators to monitor (i.e. indicator *E. coli* and enterococci), and when discussing the findings and their use as a basis for recommendations and treatment guidelines among different participants of the programme. Furthermore, development and definition of strategies and action plans to reduce AMR on both sides are also done in collaboration in the working- and steering-group, where actors and stakeholders, respectively, from the participating institutions are represented.

However, there has always been the wish to get a more in depth understanding of the potential relationship between the veterinary, food-producing and human sector, concerning antimicrobial usage (AMU) and development of antimicrobial resistance (AMR). To be able to foresee if changes in one sector will have a potentially significant impact on the other sector, it requires knowledge of the possible routes of transmission and the size and speed of transfer. 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 sector.

At the EU level, an attempt to perform cross-analysis has been made since 2015 in the JIACRA reports [ECDC/EFSA/EMA 2015. EFSA Journal 13(1):4006], despite the additional challenge of jointly analysing data collected in different countries. At the national level, even in a country such as Denmark with a long-established detailed monitoring system based on stable data delivery of high quality, there are a number of 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, with varying sampling strategies and magnitudes.

This chapter is a first attempt to cross-analyse antimicrobial resistance data from monitoring in livestock animals and humans in Denmark. First, it compares the phenotypic resistance profile of indicator *E. coli* strains recovered from the caecum of healthy animals at slaughter with the profile of *E. coli* strains isolated from the urine of UTI patients at primary health care. These two datasets represent the closest representation of *E. coli* occurrence in the animal and human populations at the community level. Ideally, a dataset based on samples from healthy humans should be used for these analyses, presenting the actual pool of bacteria and resistance genes circulating in the human population. However isolates of such cases are rarely available. Next, it maps the frequency of MLST sequence types and resistance genes of ESBL *E. coli* isolates recovered from livestock animals and meat and from humans with bloodstream infections. Such analysis is a visual demonstration of possible relationships between isolates of both origins, and can help identifying strains that may require more targeted genomic analyses to further investigate on possible transmission between human and animal reservoirs and vice-versa.

An integrated surveillance system is constituted by different levels and elements that regularly need to be evaluated (Aenishaenslin et al. 2021. *Front. Vet. Sci.* 8:611931). Textbox 3.1 in this chapter shows the first attempt to evaluate the level of One Health in DANMAP, by assessing to what extent AMR and AMU surveillance are integrated and facilitate the optimal impact of the program on the animal and human sectors.

3.2 Phenotypic resistance in indicator and clinical *E. coli* from livestock animals and clinical *E. coli* from UTI patients in primary health care

A zoonotic link between urinary tract infection (UTI) *E. coli* and *E. coli* from production animals and meat has been previously shown in Denmark by detecting clonal relatedness by PFGE between UTI *E. coli* from human patients and *E. coli* from broilers, pork meat and broiler meat [Jakobsen et al. 2012. *Eur J Clin Microbiol Infect Dis.* 31(6):1121-1129]. Another study [Jakobsen et al. 2011. *J Med Microbiol.* 60(10):1502-1511], compared a strain collection of *E. coli* phylogroup B2 (phylogenetic group of extraintestinal pathogenic *E. coli* (ExPEC) most often causing UTI) recovered from UTI patients, community-dwelling humans, broiler meat, pork, Danish broilers, and Danish pigs using DNA microarray analysis. That study showed a comparable frequency of virulence genes, but a varying frequency of resistance genes among all isolate origins. A recent study in Poland also found similar genetic background for virulence factors, and the same phylogenetic groups among avian and human ExPEC, including UTI strains [Sarowska et al. 2019. *Gut Pathog.* Feb 21;11:10], while in the USA, a study comparing *E. coli* isolated from fecal samples of healthy humans with those from UTI patients by MLST showed that the primary reservoir of UTI strains may reside outside of the human intestine [Matsui et al. 2020. *Microbiologyopen* 9(6):1225-1233].

Despite numerous findings suggesting a zoonotic link, the challenge of establishing the foodborne origin of a UTI *E. coli* remains, due to the lag between colonization of a human patient with a foodborne *E. coli* strain and the development of a UTI infection. Depending on the length of that lag, the antimicrobial resistance (AMR) profile of a UTI strain acquired by zoonotic transmission may resemble the AMR profile found at the primary animal reservoir or deviate from it. This study compares the AMR profiles obtained by antimicrobial susceptibility testing of UTI *E. coli* strains recovered from humans and strains recovered from animals, in Denmark.

We gathered a dataset containing a total of 1,370 *E. coli* isolates collected in Denmark in 2021, which are among the data reported in chapters 6, 7 and 8 of the present DANMAP report. Data included 399 isolates recovered from animals and 971 isolates recovered from humans. Isolates recovered from animals included indicator *E. coli* from caecal content collected at slaughter from healthy- broilers (34), calves <1 year of age (109) and fattening pigs (65), as well as isolates of hemolytic- (103) and non-hemolytic (88) *E. coli* recovered from clinical samples (of various kinds) collected from sick pigs. The 971 clinical isolates from humans were recovered from UTI patients at primary health care, and represent a random sub-sample of the total number of *E. coli* isolates recovered from such patients in 2021 (n= 99,077). Sub-sampling was performed in order to achieve a better balance in terms of dataset composition regarding the origin of the isolates.

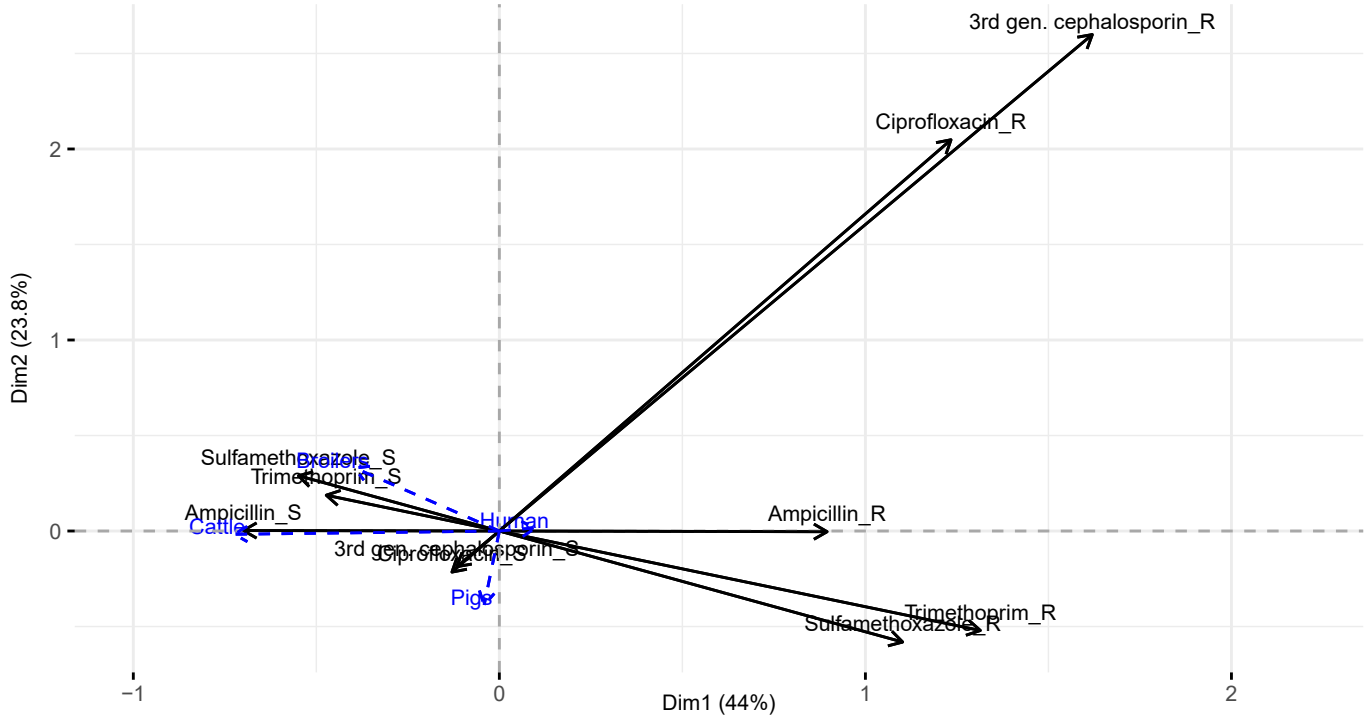
For each isolate, the data contained binary results (sensitive/resistant) of antimicrobial susceptibility testing for a selection of antibiotics that overlapped between the antibiotic panels tested in both sectors. This selection included ampicillin, ciprofloxacin, sulfamethoxazole, trimethoprim and a 3rd generation cephalosporin (cefotaxime and cefotaxime/cefpodoxim for isolates of animal and human origin, respectively). Additionally, a nominal variable indicated the origin of the isolate.

The analysis was performed in two independent steps. First, by including all isolates from humans and all indicator *E. coli* from animals. Next, by including all isolates recovered from humans and all isolates recovered from pigs, including indicator and clinical isolates.

A multiple correspondence analysis (MCA) was performed in order to assess the clustering patterns of resistance and sensitivity to different antibiotics and of different isolate origins. Analyses were performed using R statistical software version 3.6.1 [R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org]. MCA was performed with the R package *FactoMineR* version 2.4, and MCA loading plots produced with the R package *factoextra* version 1.0.7. Loading plots show how strongly each individual antibiotic resistance or sensitivity influences a principal dimension (*Dim1 and Dim2*), and the isolate origin was set as a supplementary variable to illustrate the clustering of observations of different origins.

Figure 3.1 Multiple Correspondence Analysis with indicator *E. coli* recovered from healthy animals at slaughter and *E. coli* recovered from UTI human patients

DANMAP 2021



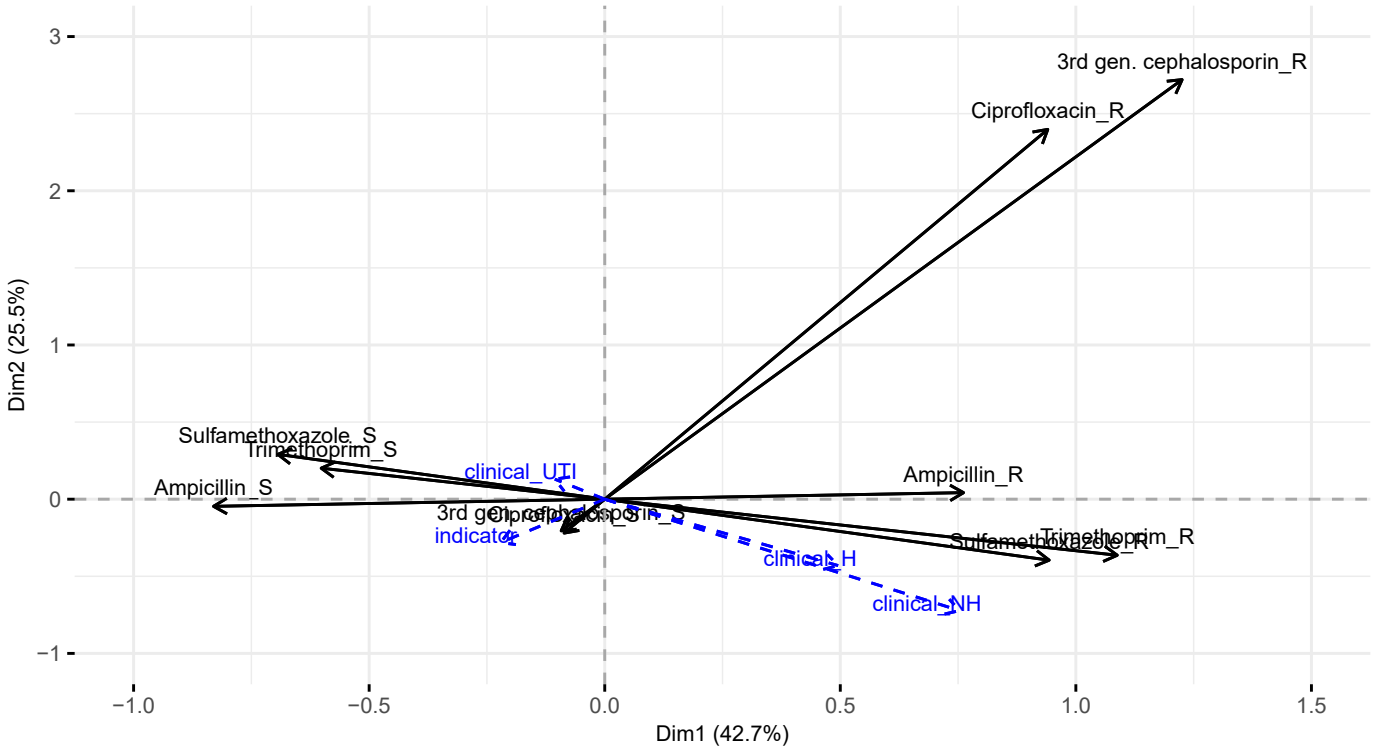
The loading plot shows the coordinates of the explanatory variables (phenotypic resistance profile to five different antibiotics) and the clustering of samples of different origins (Human, Broilers, Cattle and Pigs) in the ordination space of the two first principal dimensions (Dim1 and Dim2) These two dimensions jointly explain a total of 67.8% of variation in the data.

The *dimdesc* function from package *FactoMineR* was used to statistically assess the relationship of *DIM1 and DIM2* with the different variables.The first MCA analysis (Figure 3.1) showed a clear separation along *Dim1* between resistance and sensitivity to all antimicrobials, and some degree of separation between sample origins. All antibiotics showed a significant relation (R^2) with *Dim1*, with resistance (*R*) and sensitivity (*S*) clustering on the positive and negative coordinate space, respectively. Sample origin showed a low but significant R^2 in relation to *Dim1* (R^2 = 0.06), including all classes except *Pigs*. Observations of indicator *E. coli* from cattle and broilers located on the negative coordinate space, while UTI isolates from humans clustered on the positive space. This shows that UTI *E. coli* and indicator *E. coli* from broilers and cattle have dissimilar resistance profiles, with the first presenting overall higher levels of resistance and the latter overall higher levels of sensitivity. *Dim2* was significantly associated with all antibiotics except ampicillin, as well as to the sample origin *Pigs* (R2 = 0.01). Antibiotic variables separated along *Dim2*, by clustering resistance to 3rd generation cephalosporins and ciprofloxacin on the positive coordinate space and resistance to trimethoprim and sulfamethoxazole, and observations from pigs, on the negative space. Trimethoprim and sulfamethoxazole are antibiotics commonly used in the treatment of pigs, thus it is not surprising that resistance to these antibiotics are associated with isolates of such origin. However, in humans the usage of both trimethoprim and sulfamethoxazole has decreased markedly over the last decade and for both drugs the consumption in individuals under the age of 50 is nearing zero. Both antibiotics are used in the treatment of UTI, sulfamethoxazole formerly known as a drug to be used primarily by young women for non-recurring uncomplicated UTI, trimethoprim to be used primarily for treatment of uncomplicated UTI in the elderly above 65 years. The low levels of resistance towards trimethoprim and sulfamethoxazole in clinical human samples in the plot clearly mirror these low trends in usage among humans in Denmark.

Of no surprise is the resistance towards ciprofloxacin and 3rd generation cephalosporins, which primarily presents itself associated with human samples. Although usage of ciprofloxacin is restricted and 3rd generation cephalosporins were never introduced into the human primary sector, the human health sector is still the main area where usage of these two drugs takes place.

Similarly to the first analysis, the second MCA (Figure 3.2) also showed a clear separation along *Dim1* between resistance and sensitivity to all antimicrobials, with resistance (*R*) and sensitivity (*S*) clustering on the positive and negative coordinate space, respectively. Furthermore, this MCA separated along *Dim1* clinical isolates from pigs (hemolytic and non-hemolytic strains) from UTI isolates from humans (significant R^2 = 0.07 for sample origin, excluding the class *indicator E. coli* from pigs), with the first clustering towards resistance and the

Figure 3.2 Multiple Correspondence Analysis with indicator *E. coli* recovered from healthy pigs at slaughter, clinical haemolytic and non-haemolytic *E. coli* recovered from sick pigs and *E. coli* recovered from UTI human patients DANMAP 2021



The loading plot shows the coordinates of the explanatory variables (phenotypic resistance profile to five different antibiotics) and the clustering of samples of different origins (clinical_UTI, indicator, clinical_H and clinical_NH) in the ordination space of the two first principal dimensions (Dim1 and Dim2). These two dimensions jointly explain a total of 68.2% of variation in the data.

latter towards sensitivity. This suggests that clinical *E. coli* isolates recovered from pigs, which include isolates from several types of infection, have overall higher levels of resistance than UTI *E. coli* from humans. The second principal dimension showed again a significant association to all antibiotics except ampicillin, with clustering of resistance to 3rd generation cephalosporins and ciprofloxacin on the positive coordinate space and resistance to trimethoprim and sulfamethoxazole on the negative space. Additionally, *Dim2* showed a significant R^2 for sample origin (R^2 = 0.07), especially for the classes *clinical_UTI* (R^2= 0.23) and *clinical_NH* (non-haemolytic clinical isolates from pigs) (R^2 = -0.20). This result suggests a higher occurrence of resistance to the critically important antibiotics among human UTI isolates, compared to a higher occurrence of resistance to sulfamethoxazole and trimethoprim among non-haemolytic clinical isolates from pigs.

While several studies, using different microbiological methods, have found indication of a possible zoonotic transmission of *E. coli* from livestock animals to UTI patients, the resistance profiles of *E. coli* strains recovered from the different populations have shown less overlap. Using antimicrobial susceptibility results, the present findings did not indicate a zoonotic link and are in agreement with previous studies by suggesting that antimicrobial resistance of *E. coli* strains at the animal and human UTI patient levels are most likely led by antimicrobial consumption within each population. Further studies are needed in Denmark in order to investigate the association of antimicrobial consumption and phenotypic resistance within and across populations.

Ana Sofia Ribeiro Duarte
For further information: asrd@food.dtu.dk

3.3 Genotypic comparison of ESBL *E. coli* from livestock animals, meat and human bloodstream infections

There has been decreasing numbers of extended spectrum beta-lactamase *E. coli* (ESBL Ec) bloodstream infections (BSIs) in humans in Denmark since 2019 (see section 8.3.1), and a significant reduction in ESBL Ec has been observed in Danish broilers and broiler meat. Mughini-Gras, et al. [Mughini-Gras, et al. 2019. *Lancet Planet Health* 3(8):e357-e369] found that the primary source of community acquired ESBL Ec was through human-to-human transmission, although transmission to and from non-human sources was also evident. Other studies [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 transmissions, underlining the importance of surveying the possibility of zoonotic transfer of resistance from animals to humans.

The objective was to compare the multilocus sequence types (MLST) and ESBL-genes between humans, production animals and meat to identify any major overlaps between sectors – indicating a zoonotic link or transmission of resistance genes.

We gathered a dataset of 1,457 ESBL isolates from humans and animals from 2018 through 2021. The 884 human isolates were clinical isolates from infections sent voluntarily to the SSI reference laboratory for antibiotic resistance from the departments of clinical microbiology. The animal isolates (broiler meat: 196, broilers: 82, cattle: 51, beef: 40, pigs: 156 and pork: 48) stem from the mandatory screening programme from healthy animals and meat products. See chapter 7 and 8.3.1 for more information.

Each isolate has been sequenced as part of the surveillance and the multilocus sequence type and ESBL-gene were extracted from the sequences. All data handling was done in Python 3.8.10 and the plotly package version 5.9.0 was used to make the Sankey diagram. For the purposes of this report, only flows of five or more isolates are shown on the Sankey diagram.

Limited overlap was found in both STs and ESBL genes from humans vs. animals and food (Figure 3.3). In accordance with formerly observed overlaps (see DANMAP 2015, Textbox 7.3). ST23 was found in both humans and pigs. Likewise, ST38 was found in both humans and broiler meat. For both sequence types the ESBL genes detected differed between human and animal strains. The pig isolates from ST23 harboured C-42T mutations, whereas the human isolates harboured CTX-M-14. The broiler meat isolates from ST38 were of the CMY-2-kind, whereas the human isolates carried mainly CTX-M-14 and CTX-M-14b. Only CMY-2 and CTX-M-1 were found in both humans and food production animals or food, but not in high abundance. ST131 was responsible for roughly 50% of the ESBL-bacteraemia cases in humans, usually accompanied by a CTX-M-15 gene. ST2040 was found exclusively in broilers or broiler meat and only carried the CMY-2-gene. In general, sequence types seem to associate with species, whereas there is more variation with combinations of sequence types and ESBL-genes.

In the DANMAP 2018 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 a clonal relationship. A One Health compartmental analysis over a three-year period from Réunion [Miltgen, et al. 2022. *J Antimicrob Chemother* 77(5):1254-1262] investigated transmission of ESBL-Ec from humans, animals and environment to human colonization and infection. The study found little evidence of transmission and suggested that focus should be primarily on preventing human-to-human transmission. Conclusively, it remains challenging to find clear evidence of zoonotic transmission of ESBL Ec, even though the animal and food sectors are

potential reservoirs and possibly have a role in the introduction of ESBL Ec into the human sector, as detailed by Mughini-Gras, et al., 2019. Thus, it remains important to monitor the occurrence of ESBL Ec in humans and animals, as part of an integrated antimicrobial resistance surveillance program.

Mikkel Lindegaard
For further information: ldd@ssi.dk

Figure 3.3 A Sankey diagram comprised of 803 ESBL-isolates from humans, animals and food showing the relationship between the isolates' source, sequence type and ESBL-gene. The flows between nodes are coded according to source. Only flows of five or more isolates are shown to limit clutter. An interactive version of this figure without filtering can be found on www.danmap.org

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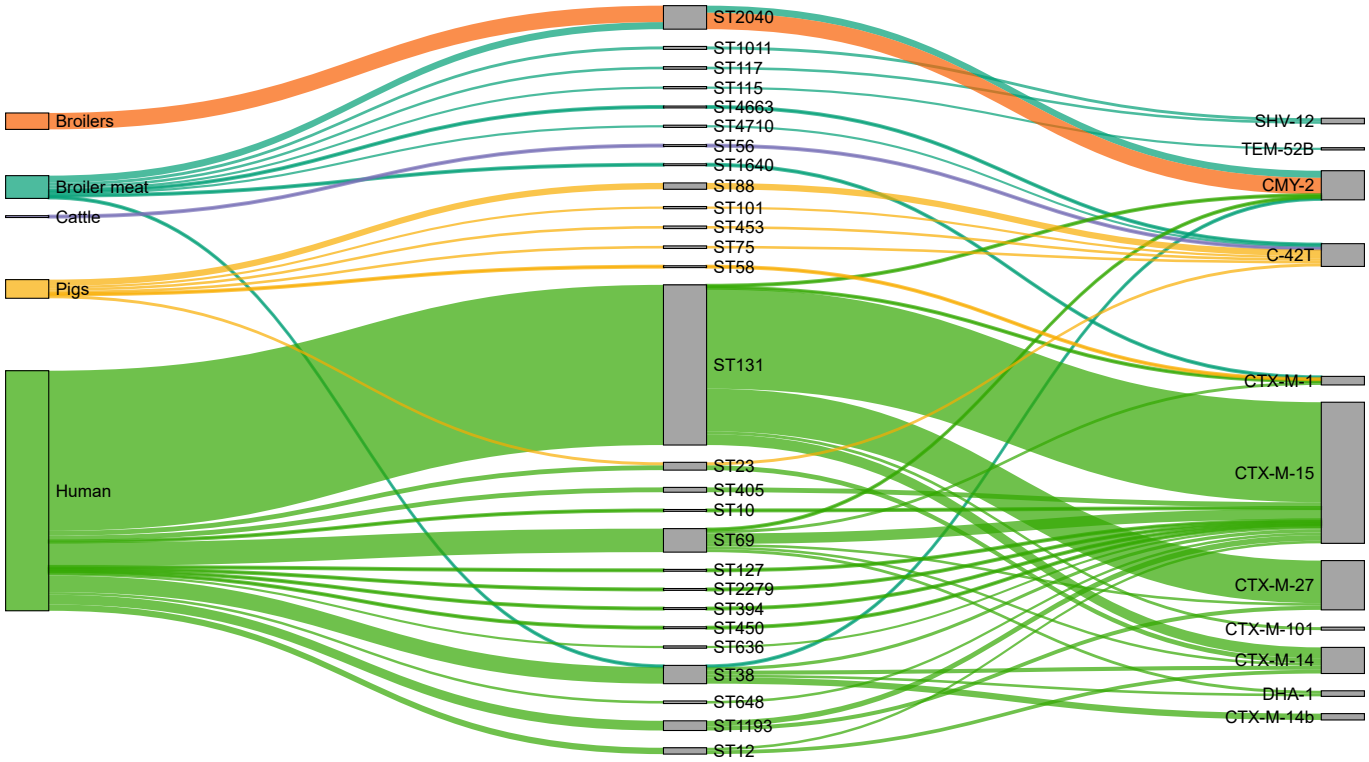


Concluding remarks

This new One Health chapter presents recent work DANMAP has done towards integrated analyses of surveillance data for antimicrobial resistance (AMR) from the human and animal/food sectors.

Two studies analyse phenotypic and genotypic AMR resistance profiles using national data for *E. coli* isolates from humans and livestock animals/meat, respectively. The comparison of phenotypic resistance profiles of indicator *E. coli* from livestock animals with those of urine isolates from UTI patients in primary health care suggests that resistance patterns found in *E. coli* isolated from human urine are associated with patients' AMU rather than with AMR patterns found in isolates from livestock animals, which again are driven by AMU in livestock production. Genomic analysis of ESBL *E. coli* isolates from livestock animals, meat and human bloodstream infections also suggests limited overlap between the sources with regards to sequence type and ESBL-genes. These findings seem to indicate that efforts to prevent transmission of AMR *E. coli* infections between sectors are currently successful in Denmark but warrant continued monitoring. Extension of these analyses to other pathogens should be explored.

As a recent evaluation of DANMAP (Textbox 3.1) also shows, the One Health approach has been a pillar of AMR and AMU surveillance in Denmark since the start of the programme and is based on high quality data and strong stakeholder engagement. In order to further strengthen preparedness and detect AMR outbreaks and transmission across sectors, more timely and routine comparison of surveillance data from both the human and animal sectors could be explored. New schemes, such as the surveillance of AMR in pathogenic bacteria from livestock (see Chapter 6), could facilitate such initiatives.

Furhtermore, it should be discussed how environmental aspects to spread of AMR through a highly intensified food production system could be investigated and included.

Ana Sofia Ribeiro Duarte, Mikkel Lindegaard, Berit Muller-Pebody and Ute Wolff Sönksen
For further information: uws@ssi.dk

Textbox 3.1

Evaluating the "Onehealth-ness" of DANMAP

Background

According to the One Health High-Level Expert Panel (OHHLEP) of the World Health Organization (WHO), "One Health (OH) is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems. It recognizes that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent". One Health has always been a pillar of the DANMAP program and in 2017 with the release of the Danish National One Health Strategy Against Antibiotic Resistance, this position has been reaffirmed.

Given the sheer complexity of designing and operating a multi-sectorial national-scale hazard surveillance program, the need to evaluate the "Onehealth-ness" of the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) has been recognized. Evaluation using the newly developed OH-EpiCap tool was performed to identify components that could be further improved in future developments of the DANMAP program.

DANMAP was evaluated considering the spread of AMR as monitored in clinical isolates from humans and indicator bacteria from food producing animals as the main hazard under surveillance and the prevention of potential risks connected to AMR that might affect both humans and animals as the objective of the program. It is necessary to clarify that in this evaluation, we tried to assess the surveillance components of the program as a whole, except for the management/execution part, which was focused on the animal sector. OH-EpiCap is under development by the MATRIX consortium, which is funded by the One Health European Joint Programme (https://onehealthejp.eu/). This textbox reports a preliminary work that will later be further developed and published by the authors. In the coming scientific paper, an overview of the OH-EpiCap tool will be presented and evaluated by using case studies from different countries as part of the CoEvalAMR network (https://coevalamr.fp7-risksur.eu/).

Materials and Methods

The OH-EpiCap aims to facilitate the identification of opportunities to improve the "Onehealth-ness" of collaborations in the surveillance of a hazard. The OH-EpiCap tool is composed of three thematic domains (Dimensions), each with four different targets that are segmented into four questions, for a total of 48 standardized indicators, which are briefly presented in Table 1. These questions are intended to be answered using a semi-quantitative scale from 1 to 4, with 4 representing the ideal scenario, in most cases.

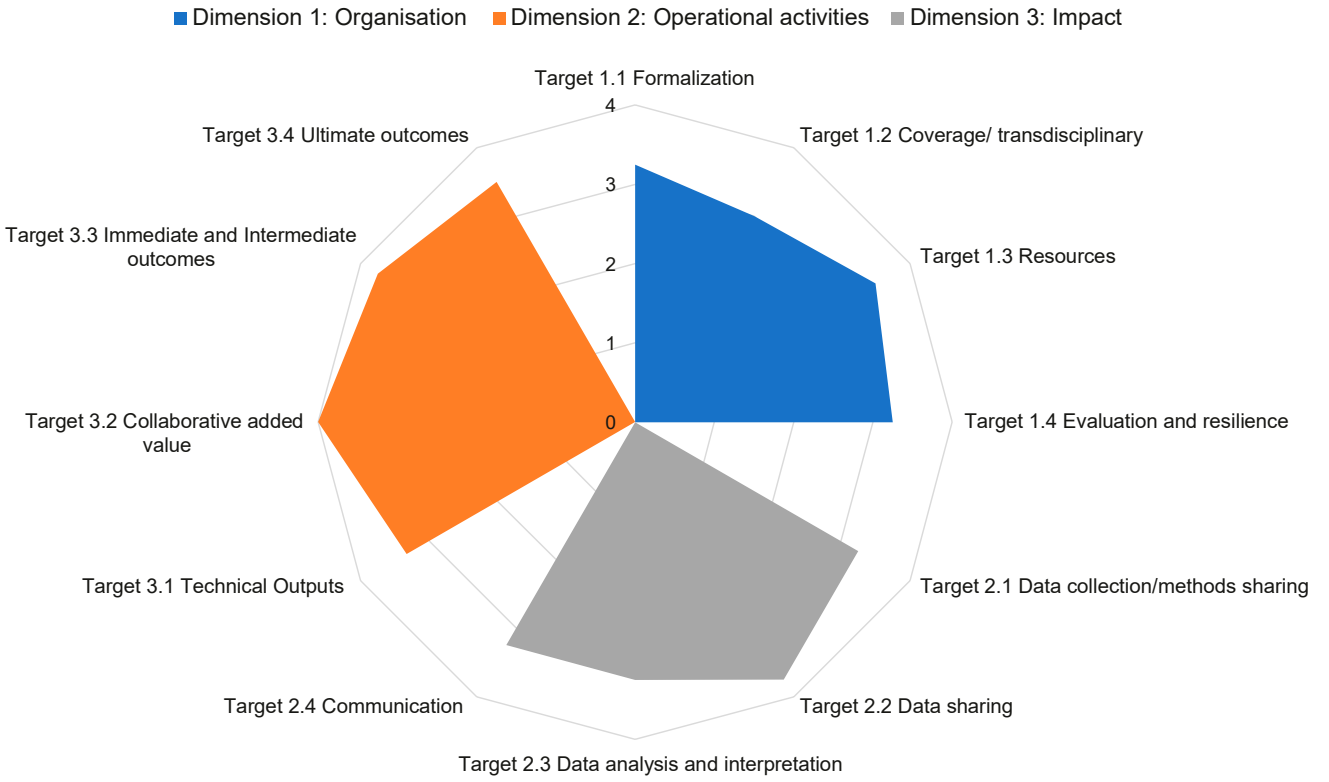
Table 1 Dimensions, targets and topics evaluated by the OH-EpiCap tool. (Rephrased after the flyer OH-EpiCap: evaluation tool for One Health epidemiological surveillance capacities and capabilities, available at: https://onehealthejp.eu/) DANMAP 2021

Dimension 1: Organisation			
Target 1.1 Formalisation: common aim, support documentations, shared leadership, and definition of roles/composition of coordination committees.	**Target 1.2 Coverage:** inclusion of all relevant actors, disciplines, sectors, geography, populations, and related hazards.	**Target 1.3 Resources:** budget and human resources, program training, and sharing of resources.	**Target 1.4 Evaluation and resilience:** internal and external evaluations, development/ implementation of corrective measures, and adaptability to change.
Dimension 2: Operational activities			
Target 2.1 Data collection and methods sharing: multisectoral collaboration in the design of surveillance protocols and data collection, harmonization of laboratory techniques and data warehousing.	**Target 2.2 Data sharing:** data sharing agreements, assessment of data quality, usefulness of shared data, and the compliance of data with the FAIR (findability, accessibility, interoperability, reusability principle.	**Target 2.3 Data analysis and interpretation:** multisectoral integration for data analysis, sharing of analysis techniques, sharing of scientific expertise, and harmonization of indicators.	**Target 2.4 Communication:** internal and external communication, dissemination to decision-makers, and information sharing in case of suspicion/ particular events.
Dimension 3: Impact			
Target 3.1 Technical outputs: timely detection of emergence, epidemiological knowledge improvement, increased effectiveness of surveillance, and reduction of operational costs.	**Target 3.2 Collaborative added value:** strengthening of the OH team and network, international collaboration, and common strategy (road map) design.	**Target 3.3 Immediate and intermediate outcomes:** advocacy, awareness, preparedness, and interventions based on the information generated.	**Target 3.4 Ultimate outcomes:** research opportunities, policy changes and behavioural changes and better health outcomes.

continued ... Textbox 3.1

The first version of the OH-EpiCap was filled in at a meeting between representatives from the DANMAP management, academia, and the Danish livestock industry. The answers given were sent to other relevant stakeholders, who agreed and commented on the answers given.

Figure 1: Average score of DANMAP in the target areas covered by the OH-EpiCap tool DANMAP 2021



Results and Discussion

A graphical outcome of the OH-EpiCap evaluation of DANMAP can be seen in Figure 1. The program scored highly in every dimension, averaging above 3 in all target areas, demonstrating a high level of "Onehealth-ness". Overall, when filling in the OH-EpiCap tool a conservative approach was chosen. Hence, when in doubt between two options, a lower score was chosen to raise awareness and promote discussion around those target areas. The following points were identified as relevant for discussion.

Dimension 1: Organisation

Main points of critique identified in the interview were three regarding organisation: 1. All relevant supporting documentation to DANMAP should preferably be compiled and shared at one common digital point, increasing the public accessibility. 2. The steering committee does not include all the sectors that potentially are relevant to OH surveillance, as the environment is not represented. 3. All actively engaged sectors are represented in the program's coordination group. However, more representatives from the livestock industry and the environmental sector could reinforce the OH approach.

Previous national research performed around the shift of the sequel have led to the non-inclusion of environmental data into the surveillance programme. Since then, animal production and hospital activity have markedly intensified, and AMR potentially spreading through wastewater or manure have been mentioned as possible critical observation points for future inclusion into AMR surveillance. The needed data, methodologies, and analyses are currently being considered. Over time, the program has evolved, adapting to new challenges, and optimizing content and processes, some of it following the conduction of regular internal evaluations. Yet, evaluations could have been performed using a more standardized methodology, easing the implementation of corrective measures proposed in a timelier way.

For the current aims of the program, economic and human resources are sufficient and sustainable. However, to investigate emerging issues, adapt and include them in the surveillance program, an extension of the budget would be required to get more staffing resources and analytical means. This is also the case for addition of new components to the surveillance program, e.g. environmental aspects.

Dimension 2: Operational activities
Harmonisation of indicators across sectors and methodology for sampling the animal population for AMR surveillance could possibly be improved but would inevitably come with budget challenges. Joint multi-sectorial analysis could potentially be improved in the future. Transparency, communication, and accessibility of data need to be constantly evaluated, given that these change when the methodology changes or the aim of the program is altered. Investment in further training of professionals responsible for prescribing and using antimicrobials could also be considered.

Dimension 3: Impact
OH surveillance has always been the basis of DANMAP, therefore questions regarding the added value of adapting to a OH response were not considered relevant. Given the more than 25 years of the program, its impact on epidemiological knowledge are clear and have guided sector interventions and policy changes. In Denmark, there is a strong will of working collaboratively, OH networks are well-functioning and the level of awareness among the stakeholders is very high, even if translation into action could be further improved.

Pedro Moura, Ute Wolff Sönksen, Lis Alban and Marianne Sandberg
For further information: pedrojoaomouravet@gmail.com

Textbox 3.2

Update from International Centre for Antimicrobial Resistance Solutions (ICARS)

ICARS offers tailored support to low-ressource settings for the development and implementation of context-specific AMR solutions, contributing to each country's National Action Plan and presenting an opportunity for scale-up, cross-country, and cross-regional learning.

Why ICARS?

While much research has revealed successful solutions for tackling AMR, there is still a critical gap in translating this evidence into action. Even though many countries have developed AMR National Action Plans (NAPs), in resource poor settings the challenge remains for how best to prioritise and implement interventions to reduce AMR. Furthermore, the growing rates of drug-resistant microorganisms detected in animals, humans and the environment are evidence that a siloed approach is not enough, and global efforts to address AMR should span the One Health spectrum.

What does ICARS do?

Using simultaneous top-down and bottom-up approaches, ICARS partners with low-and middle-income ministries and their local research institutions, to co-develop cost-effective solutions to tackle AMR. Each solution, informed by intervention and implementation research, is tailored to tackle a local AMR challenge and advance NAP implementation. To deliver long lasting change and to avoid duplication, ICARS collaborates with a range of stakeholders to build on existing national and international initiatives, and aiming to boost investments and efforts across sectors.

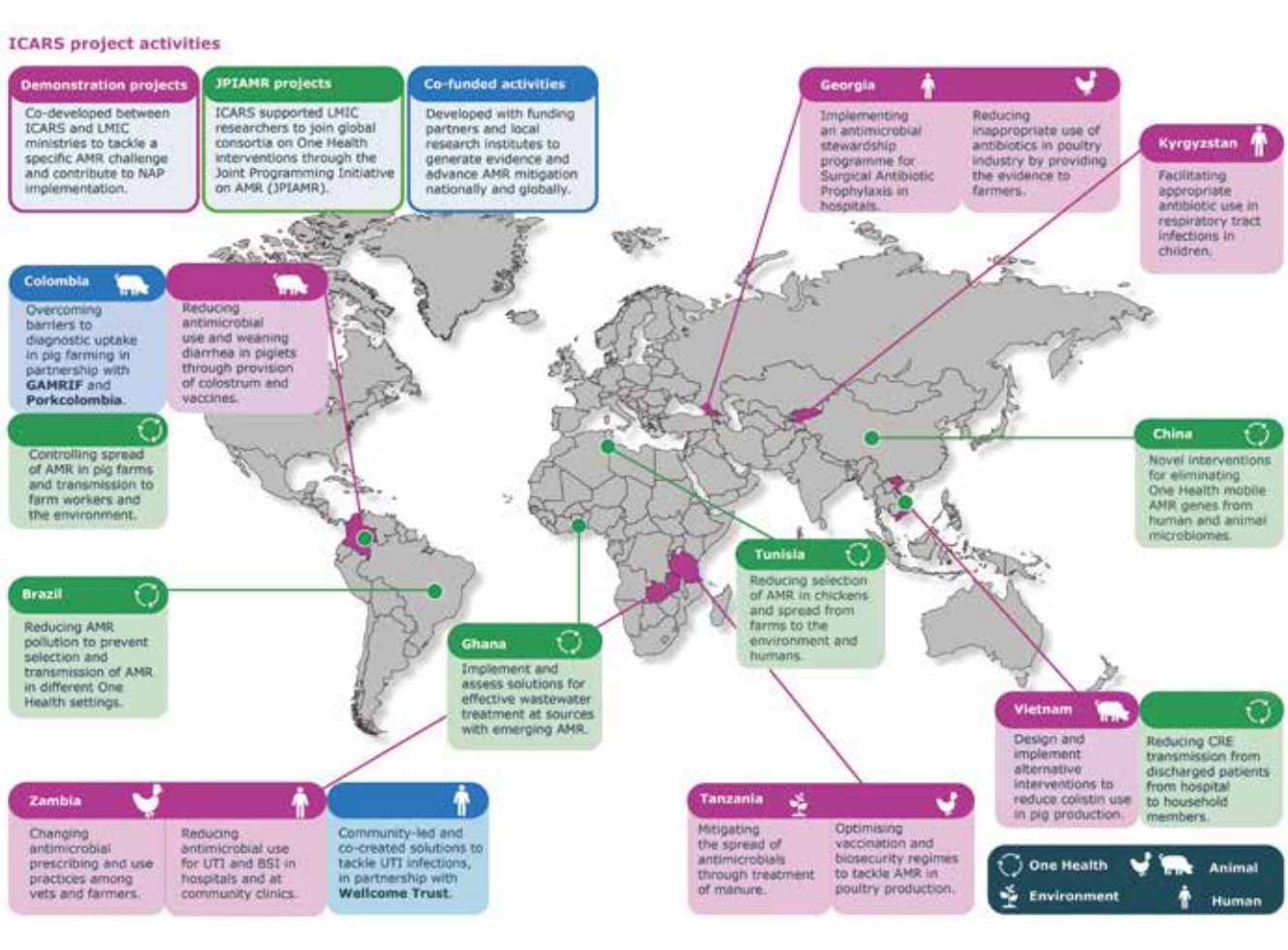
The ICARS strategy is based on the following interconnected strategic pillars:

- Pillar 1: Develop and test context-specific solutions for AMR mitigation
- Pillar 2: Support the translation and uptake of existing evidence and innovation into policies, programmes and practice
- Pillar 3: Advocate for context-specific, country-owned AMR mitigation solutions
- Pillar 4: Support targeted capacity and capability building
- Cross cutting pillar: A trustworthy partner and platform for delivering country-owned AMR solutions

ICARS research project portfolio

In 2021, ICARS began implementing its first two demonstration projects in Georgia and Vietnam, in the human and animal sector respectively. In 2022 a further seven demonstration projects across Latin America, Africa and Asia the One Health spectrum have been agreed upon and signed. This milestone follows exchange between ICARS research advisors, country ministries and local research institutions, discussing and developing a range of interventions that tackle specific AMR challenges.

In addition to co-developing projects with country ministries, ICARS has also begun collaborations with national and international organisations on a range of supporting actitivities to tackle AMR. For example, last year ICARS and the UK Global AMR Innovation Fund (GAMRIF) joined hands to foster and support an innovative project led by Porkcolombia to improve uptake of disease diagnostics at pig farms in Colombia. With funding from ICARS and GAMRIF, interdisciplinary experts on the ground are assessing the challenges and opportunities around the uptake of diagnostic veterinary services in Colombia, and their potential impact on the reduction of antibiotic use in farming. Results from the pilot (published later this year) will serve as an example for other countries to consider how to improve access to veterinary diagnostics to improve sanitary status of farms, increase understanding of disease occurrence and provide a variety of information on herd health and the different pathogens that cause swine diseases.



We are also working in partnership with multiple stakeholders including the World Health Organisation (WHO), the Global AMR R&D Hub, The British Society for Antimicrobial Chemotherapy (BSAC), the International Livestock Research Institute (ILRI) and ReAct on a range of activities that strengthen existing demonstration projects or compliment other in-country initiatives.

Governance

Initiated by the Danish government in 2019, ICARS became an independent organisation in 2021 governed by an international Board of Directors and informed by a Technical Advisory Forum. Guided by its Board, ICARS aims to partner with countries, foundations and organisations to advance drug-resistant infections mitigation in LMICs. ICARS is also supported by Funding and Mission Partners, who provide financial and in-kind contributions to advance ICARS' agenda. In 2022, Zambia and India joined ICARS as Mission Partners, a commitment that demonstrates their support to the vision and mission of ICARS and a commitment to work actively to promote the AMR agenda in their national context.

Helle Engslund Krarup, Robert Leo Skov and Sephy Valuks
For further information: contact@icars-global.org

Textbox 3.3

Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in the pre-antibiotic era: the role of hedgehogs and antibiotic-producing dermatophytes

The discovery of antibiotics more than 80 years ago instigated an era of drug innovation and implementation in human and animal health. Clinical use of these antibiotics were all followed by the rapid emergence of antibiotic resistance genes in clinical isolates of many common pathogens. This history has led to the generally accepted view that antibiotic resistance in pathogenic bacteria is a modern phenomenon driven by our use of antibiotics.

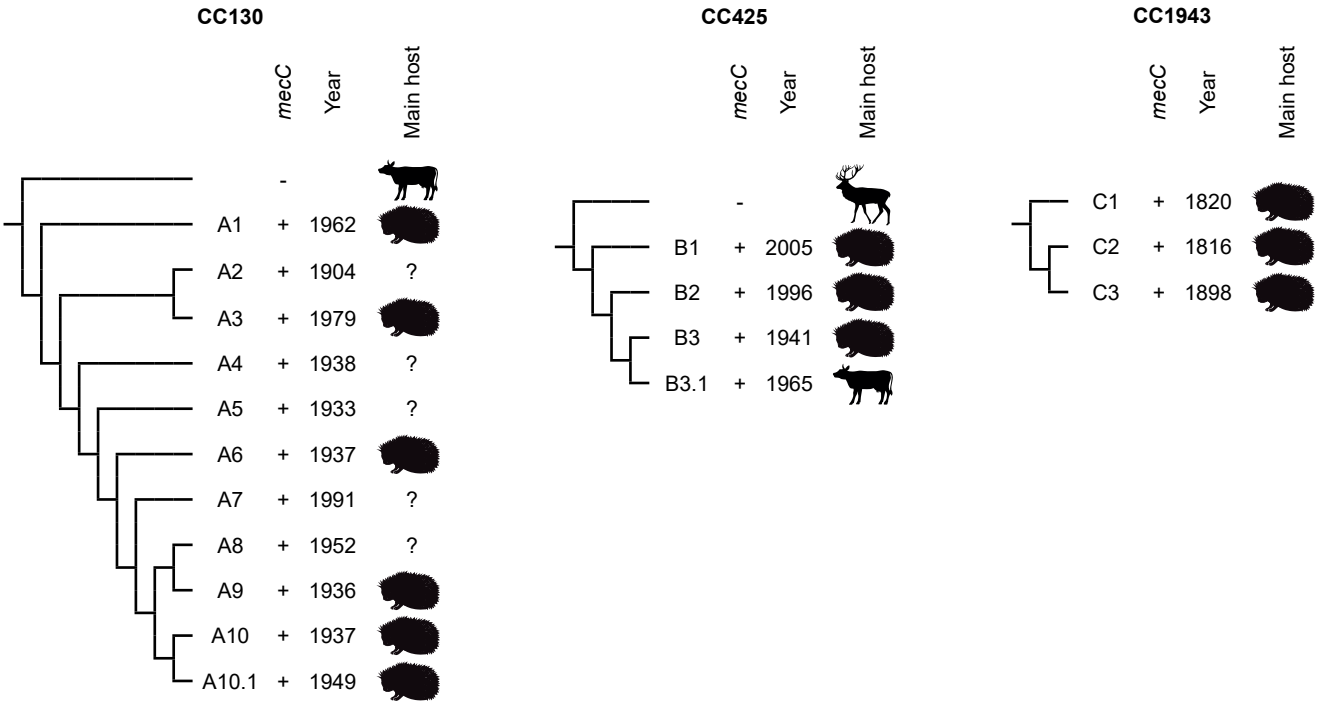
Methicillin-resistant *Staphylococcus aureus* (MRSA) was first identified in patients in 1960 just one year after the introduction of the penicillinase-stable penicillin methicillin as a therapeutic option against the rapid emergence of penicillinase-producing – and hence penicillin-resistant – *S. aureus* isolates during the 1940s and 1950s. Methicillin resistance has subsequently emerged in many *S. aureus* clones around the world as a result of horizontal acquisition of the *mecA* gene from other staphylococci, both in hospital and community settings as well as food animals such as pigs and cattle. Two recent reports from Denmark and Sweden have shown that hedgehogs are frequent carriers of particular lineages of MRSA carrying the *mecC* gene, which is a homologue of the *mecA* gene. These clones account for 3% of all human MRSA infections in Denmark and differ from other MRSA clones by being susceptible to almost all non-β-lactam antibiotics.

The detection of *mecC* carrying MRSA clones in hedgehogs prompted us to investigate the hypothesis that the emergence of these bacteria was initially driven by natural selection rather than by clinical use of antibiotics. For this purpose, we sequenced 1,127 *mecC*-MRSA and MSSA isolates belonging to CC130, CC425, and CC1943, which represent the most successful *mecC*-MRSA clones.

Bioinformatics analyses (e.g., identification of antimicrobial resistance genes and host-specific genetic markers) and construction of time-scaled phylogenies, showed that the *mecC*-MRSA isolates belonged to 16 monophyletic lineages, most of which contained hedgehog isolates, and that the earliest *mecC*-MRSA lineages appeared in European hedgehogs in the early 1800s well before the first antibiotics became widely available as therapeutic options in human and veterinary medicine in the 1940s (Figure 1).

In addition, the analyses revealed that most *mecC*-MRSA isolates from humans and food animals originate from local hedgehog reservoirs (Figure 1). Interestingly, the *mecC*-MRSA CC130 and *mecC*-MRSA CC425 lineages appear to have evolved from two distinct methicillin-susceptible *S. aureus* (MSSA) populations circulating in ruminants and wildlife (Figure 1). Analyses of the hedgehog dermatophyte *Trichophyton erinacei* revealed that it produces penicillin-like antibiotics, which provide a natural selective environment where MRSA isolates have an advantage over MSSA isolates. Our findings thus suggest that methicillin-resistance is an acquired phenotype associated with adaptation to hedgehogs. These results underscore that there are no boundaries between natural, agricultural, and human ecosystems and it is therefore necessary to look at antibiotic resistance in a One Health perspective.

Figure 1 Population structure of *S. aureus* CC130, CC425 and CC1943 DANMAP 2021



The *mecC*-MRSA isolates could be divided into 16 lineages (A1 to A10, B1 to B3, and C1 to C3). A10.1 and B3.1 refer to a Danish *mecC*-MRSA CC130 sublineage from Jutland and to a British *mecC*-MRSA CC425 sublineage carrying a genetic marker of ruminant adaptation

Jesper Larsen and Anders Rhod Larsen
For further information: Jesper Larsen, jrl@ssi.dk

References

[1] Larsen et al. Emergence of methicillin resistance predates the clinical use of antibiotics. Nature 602, 135-141 (2022)

Textbox 3.4

Clostridioides difficile – investigating genetic overlap between human and animal strains

Clostridioides difficile is a spore-forming, strictly anaerobic bacterium that is widely disseminated in a broad range of domestic and wild animal species. It shows pronounced intra- and inter-species differences in prevalence and clinical relevance, ranging from being a commensal of the gut in some and causing gastrointestinal diseases in others. In humans especially younger individuals may be asymptomatic carriers, while vulnerable recently hospitalised and/or antibiotic treated patients will be at a much higher risk of being infected with the hyper-virulent types, particularly of strains carrying the binary toxin. Since 2009, *C. difficile* of the toxin-producing "ST1 (PCR ribotype 027)" isolated from hospitalised patients is mandatory to report and national surveillance of hypervirulent strains is carried out by the Department for Foodborne Infections at Statens Serum Institut. Since 2016, a sentinel surveillance of all toxin-positive clinical samples or isolates has been performed in a collaborative effort between the clinical departments of microbiology and SSI. Samples are collected and sent during one month in spring and one month in autumn. This ensures a more real and unselected image of the type distribution in the Danish reservoir and serves to discover and record emergence of unusual types.

Recently, the numbers of *C. difficile* infections (CDI) not associated with hospitalisation have increased, indicating changing epidemiology of community associated (CA) transmissions. International studies have shown that some types are more prevalent among CA infection, in particular ST11, which has also been found in farmed animals and is considered a human hypervirulent and multidrug resistant type. In Denmark, there are approximately 5000 CDI cases each year and ST11 is the second most common type accounting for 8-10% of all human CDI cases (See Figure 1 for population structure of Danish human types).

In order to investigate possible genetic overlap between human and animal *C. difficile*, a total of 330 pig fecal samples were collected from 14 different organic farms in 2020 and cultured for *C. difficile*. Thirty-three (10%) were *C. difficile* positive and among those 21 were ST11 originating from 6 pig farms. Additionally, 184 conventional slaughterhouse pig fecal samples (2021) were cultured and among those we found seven STs (ST6, ST8, ST9, ST13, ST16, ST44 and ST45), none of them being related to human isolates.

All ST11 isolates were whole genome sequenced and sequences were compared to 390 human ST11 isolates from 2020-2021 by core genome/whole genome MLST (cgMLST/wgMLST) and single nucleotide polymorphism (SNP), while the presence of antimicrobial resistance genes (ARG) were investigated by AMRFinder (https://www.ridom.de/u/NCBI_AMRFinderPlus.html). Eighteen out of 21 veterinary ST11 were 0-2 wgMLST different from human isolates indicating either direct transmission or a shared intermediate reservoir.

The 18 pig isolates were part of three different human clusters (≤3 SNP) representing ca. 30% of human isolates, indicating that the animal clones are common among human CDI. Two clusters were ST11(RT078) (Figure 2) and one was ST11(RT066). ARG profile within each human/animal cluster, confirmed similarity and important potential of resistance (not confirmed be phenotype), (Table 1).

This study confirms previous findings, where genetic overlap was found among human and animal *C. difficile* ST11. However, the study also indicates that ST11 is restricted to certain farmed animal environments as none was found in conventional slaughter pigs. Therefore, more data are needed to pinpoint specific routes of transmission and future studies should include investigation of more samples from food, animal and environmental sources, along with geographical location of patients and farms from where similar strains are obtained.

Figure 1 Population structure obtained by minimum spanning tree of core-genome MLST (cgMLST) (BioNumerics, 1999 alleles) of major Danish clinical *C. difficile* sequence types (STs) (colored) derived from WGS data on 2788 isolates obtained from the national sentinel surveillance 2018-2022 (all toxigenic isolates collected one month in spring and fall from all Danish Dept. of clinical Microbiology). Two different ST11, i.e. RT078 and RT066 are circled on figure DANMAP 2021

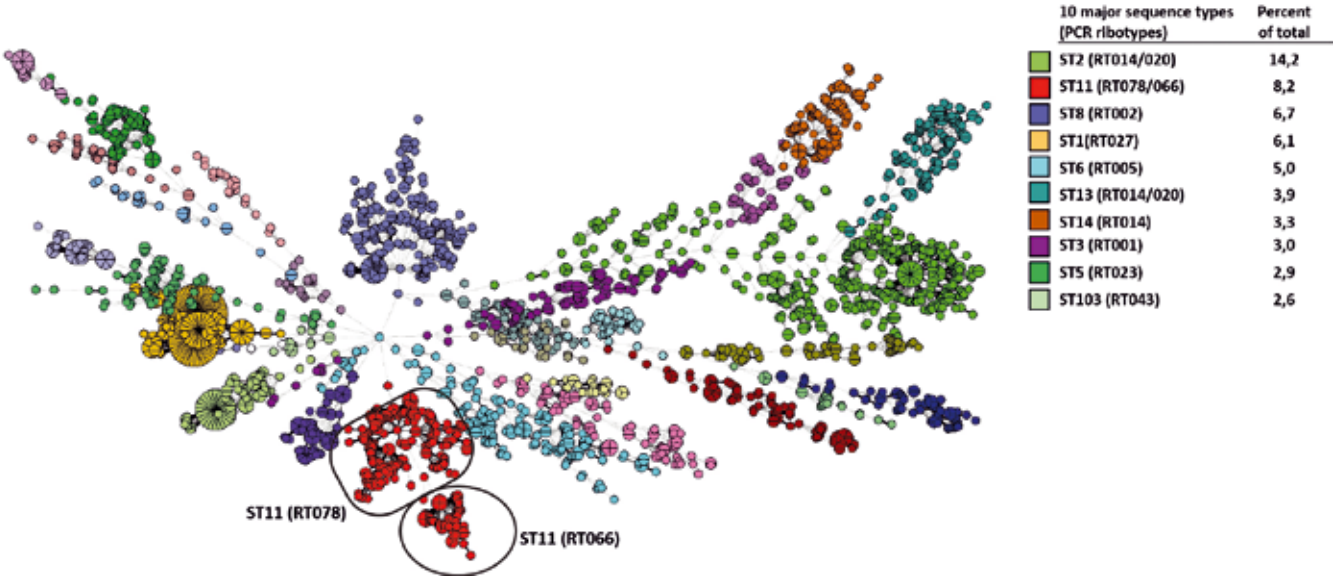
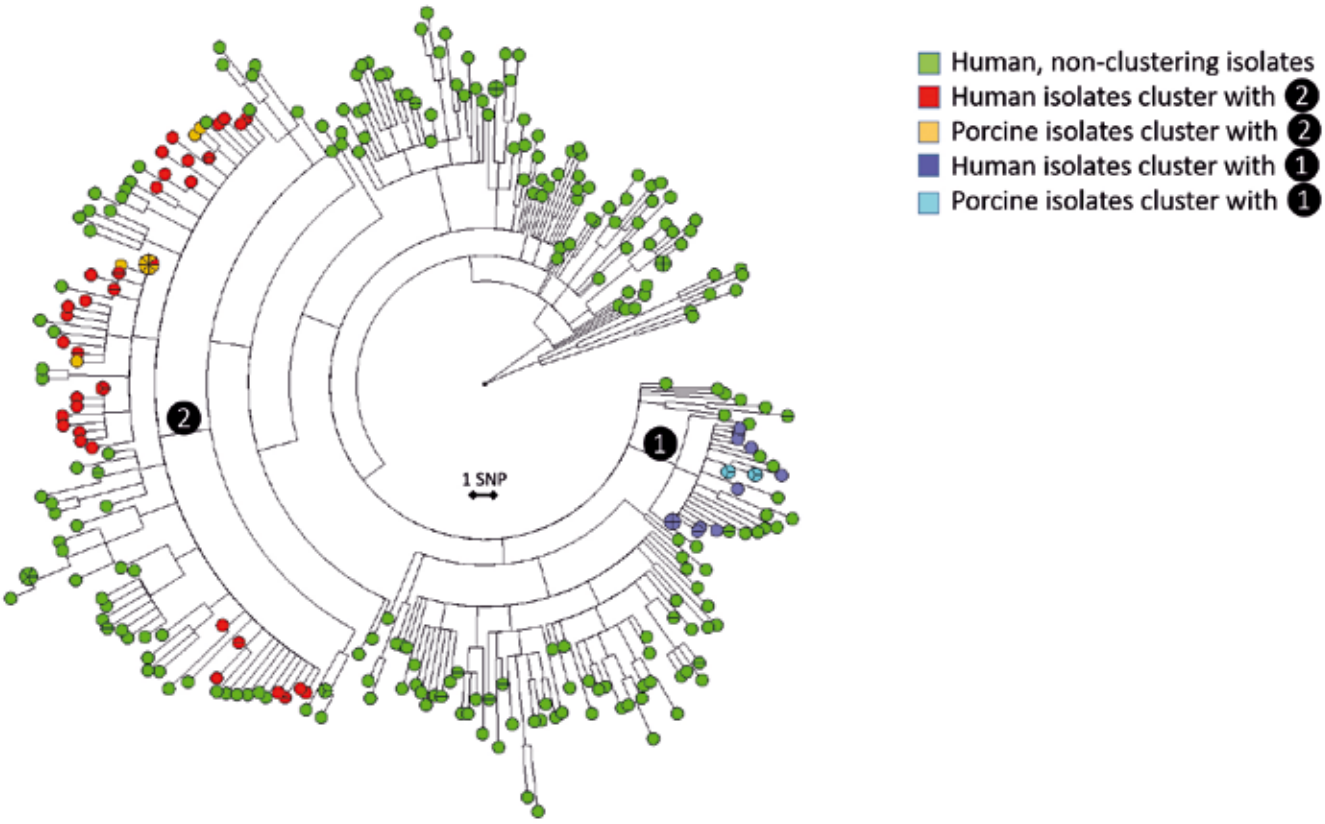


Figure 2 SNP tree (1047 SNPs) containing 308 clinical isolates from 2020-2021 (ST11, RT078) and 17 porcine isolates (2020). The two highlighted clusters are defined as ≤3 SNP differences DANMAP 2021



continued ... Textbox 3.4

Table 1 Characteristics of the three different ST11 veterinary-human clusters. Cluster 1 and 2 of ST11(RT078) are shown in Figure 2
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Cluster # (ST/RT)	No. porcine isolates (Origin)	No. human isolates (% of total human)	Major ARGs
❶ (Figure 2) (ST11/RT078)	6 (Farm #1, 2)	14 (4.5%)	β-lactam: *blaCDD* Fluoroquinolone: *gyrB* (S366V+S416A)
❷ (Figure 2) (ST11/RT078)	11 (Farm #2, 6, 8, 9, 10)	35 (11.4%)	β-lactam: *blaCDD* Fluoroquinolone: *gyrB* (S366V+S416A) Aminoglycoside: *ant(6), aph(3), sat4* Tetracycline: *tet(M)*
❸ (ST11/RT066)	1 (Farm #8)	12 (14%)	β-lactam: *blaCDD* Fluoroquinolone: *gyrB* (S366V+S416A)

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Søren Persson
For further information: SPN@ssi.dk