3

ONE HEALTH AMR

3. One Health AMR

3.1 Introduction

One Health is defined as a unified approach to optimise 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 handled and stored in separate databases by the animal and human sectors, although interpretation of results is done in cooperation. Moreover, integration happens when discussing resistance findings in common indicators (i.e. indicator *E. coli* and enterococci), using it as a basis for recommendations and treatment guidelines among different participants of the programme. Furthermore, DANMAP supports the development and definition of strategies and action plans to reduce AMR on both sides in a collaborative manner, and by fostering the dialogue between different actors and stakeholders, across sectors.

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 [JIACRA III, 2016-2018, ECDC, EFSA, EMA; 2021], 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 delivery of high quality data, 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.

Here we try to cross-analyse antimicrobial resistance data from monitoring in livestock animals and humans in Denmark. We map the frequency of multilocus sequence types (MLST) and resistance genes of ESBL/AmpC-producing *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.

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

There has been decreasing numbers of extended spectrum beta-lactamase-producing *E. coli* (here abbreviated to ESBL Ec) bloodstream infections (BSIs) in humans in Denmark since 2019 (see Chapter 8, Section 8.3.1), and a significant reduction in ESBL Ec has been observed in Danish broilers and broiler meat (see Chapter 7, Section 7.3.1) . 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 [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 transmissions, underlining the importance of surveying the possibility of zoonotic transfer of resistance from animals to humans.

The objective was to compare the MLST and ESBL/AmpC-genes between humans, food-producing animals and meat to identify any major overlaps between sectors – suggesting a zoonotic link or transmission of resistance genes.

To the data used in the analysis presented in DANMAP 2021, Chapter 3, we added new data from 2022 to comprise a dataset of 1,649 ESBL Ec isolates from humans and animals from 2018 through 2022. The 1,649 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: 145, broilers: 90, cattle: 43, beef: 32, pigs: 165, pork: 36, and turkey meat: 81) stem from the mandatory screening programme from healthy animals and meat products. See Chapter 7, Section 7.3.1, and Chapter 8, Section 8.3.1, for more information regarding the data and data collection.

Each isolate has been sequenced as part of the surveillance activities and the multilocus sequence type and ESBL/AmpC-genes were extracted from the sequences. All data handling was done in Python 3.11.0 and R statistical software version 4.2.1 and the plotly package version 5.15.0 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, which reduced the number of included isolates from 1,649 to 993 isolates.

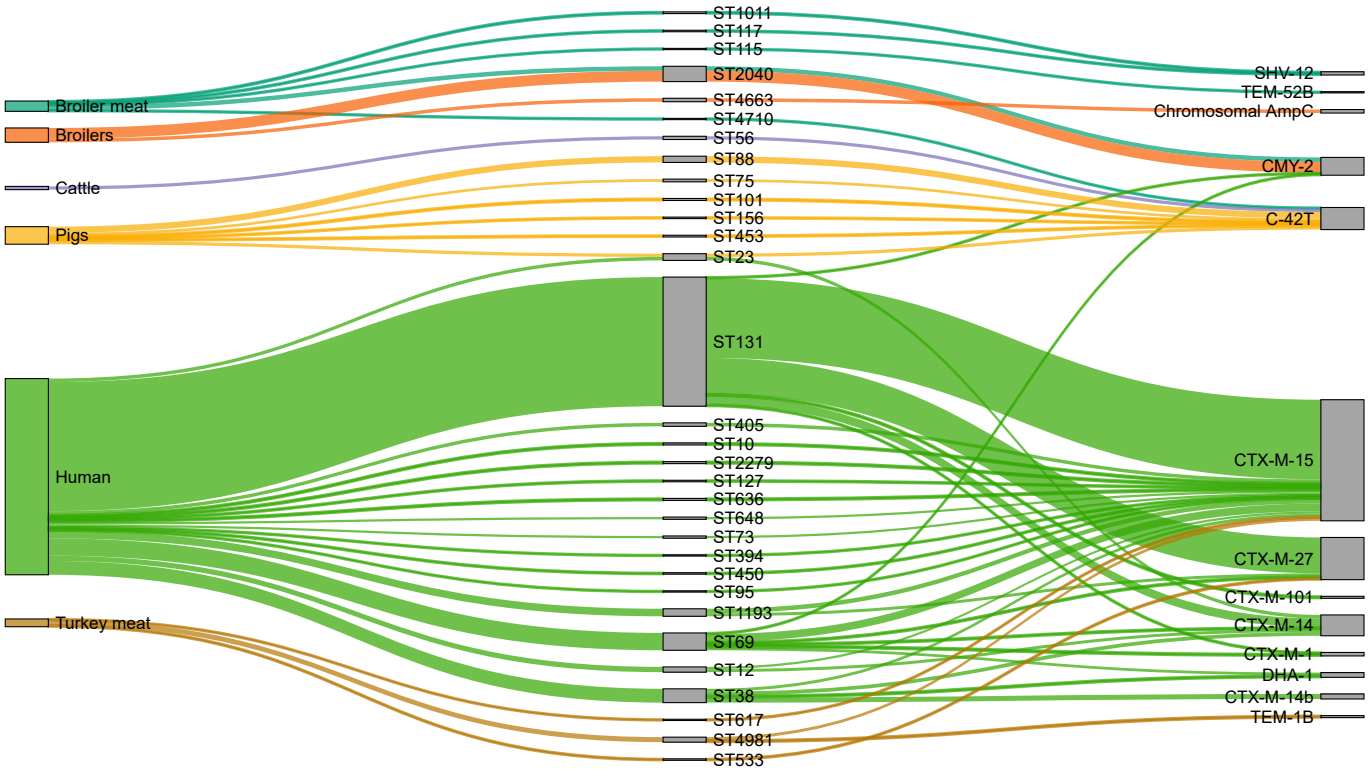
Limited overlap was found in both STs and ESBL/AmpC genes from humans vs. animals and food (Figure 3.1). The few overlaps observed were in accordance with former findings (see DANMAP 2015, Textbox 7.3): ST23 was found in both humans and pigs. Likewise, ST38 was found in both humans and broiler meat. However, for both sequence types the ESBL/AmpC 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, CTX-M-15 and CTX-M-27 were found in both humans and food-producing 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. Interestingly, turkey meat isolates in many cases also carried CTX-M-15, but differed from human isolates on sequence types. 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 variance in combinations of sequence types and ESBL/AmpC-genes.

In the 2018 DANMAP report (described in 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.

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 the 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.

Figure 3.1 A Sankey diagram comprised of 993 ESBL-isolates from humans, animals and food showing the relationship between the isolates' source, sequence type and ESBL/AmpC-gene
DANMAP 2022



The flows between nodes are coded according to source. Only flows of five or more isolates are shown to limit clutter

Conlusion and future perspectives

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

Genomic analysis of ESBL/AmpC-producing *E. coli* isolates from livestock animals, meat and human bloodstream infections suggests limited overlap between the sources with regards to sequence type and ESBL/AmpC-genes. These findings seem to indicate that efforts to prevent zoonotic transmission of AMR *E. coli* are currently successful in Denmark but warrant continued monitoring. Extension of this analysis to other pathogens should be explored. Additionally, more sophisticated analyses are in the works taking a deeper dive into the genomics of ESBL Ec with the likes of source attribution and flank analysis (see Textbox 3.1) to provide a deeper comprehension of how AMR spreads between sectors.

As this year's editorial highlights, 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 will be explored. New schemes, such as the surveillance of AMR in pathogenic bacteria from livestock (see Chapter 9), facilitate such initiatives, creating a base for further comparison of data with not only healthy but diseased animals.. Furthermore, it should be investigated how and to what extent it is relevant to monitor the occurence of AMR in the environment as to judge where the environment is an important reservoir for AMR. Likewise, it would be of interest to use these data for risk assessments and forecasts of possible spread between sectors, helping to fill the knowledge gaps about cross-sectoral AMR transmission.

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Textbox 3.1

Flankophile: A bioinformatics pipeline for prokaryotic genomic synteny analysis

Background

Antimicrobial resistance (AMR) is a serious and increasing threat to human health globally. While it is generally accepted that antimicrobial use will select for AMR, the transmission of AMR bacteria and antimicrobial resistance genes (ARGs) also shapes the occurrence of AMR. Thus, investigating, understanding and quantifying the spread of ARGs across geographical borders, between different animal and human hosts, as well as within hospitals is important for designing optimal interventions against the spread of AMR.

However, while several studies have documented the transmission of specific AMR clones globally and between hosts, it has been more difficult to determine transmission of specific ARGs. It is especially challenging to determine the epidemiological importance for human health of ARGs found in uncultivable bacterial species from environmental samples and livestock. Occasionally, the same ARGs have been found in several different reservoirs and bacterial species, but a direct transmission link can be difficult to establish.

Flankophile - a new bioinformatics tool to analyze flanking regions

Analysis of flanking region sequences can be useful when comparing mobile prokaryotic sequences from different bacterial isolates or metagenomes. A new bioinformatics tool, a pipeline called Flankophile [1], can analyze flanking regions and sequence variants. The main feature of Flankophile is that it visualizes flanking region synteny (the genetic code that comes before and after a gene) and sequence variants in publication-ready plots with distance trees, gene annotations and metadata.

The study of acquired ARGs is an obvious use case for Flankophile due to the typically diverse genetic context of the genes. To demonstrate Flankophile, we applied it to a dataset of sequenced 2,006 bacterial clinical isolates from humans (including various species recovered from different infections) and 273 faecal metagenomes from pigs in Denmark, and compared the ARGs found in each host species.

Results

Gene variant results, i.e. considering hits for unique gene variants in the reference database and not closest match hits, showed that only approximately 4% (N=42) of all unique ARG variant sequences found among all samples (N=1,052) were detected in samples from both humans and pigs. Among those ARG variants detected in both hosts, and with long enough flanking region sequences available, flanking region analysis showed multiple examples of ARGs where the entire 3,000 base pair flanking region was identical in samples from both hosts, but also examples of ARGs where no such overlap was observed. Thus, the use of Flankophile provides further resolution and better genomic evidence for zoonotic ARG transmission, by disclosing the possibility of a recent common source of an ARG variant detected in different hosts.

In addition, more than 80% of the unique ARG variants identified, including some frequently observed, were not identical to reference sequences. This suggests that there are possibly many more circulating common gene variants than the ones found in extensive reference databases, such as the ResFinder database. Thus, Flankophile is also well suited to discover and report such new ARG variants.

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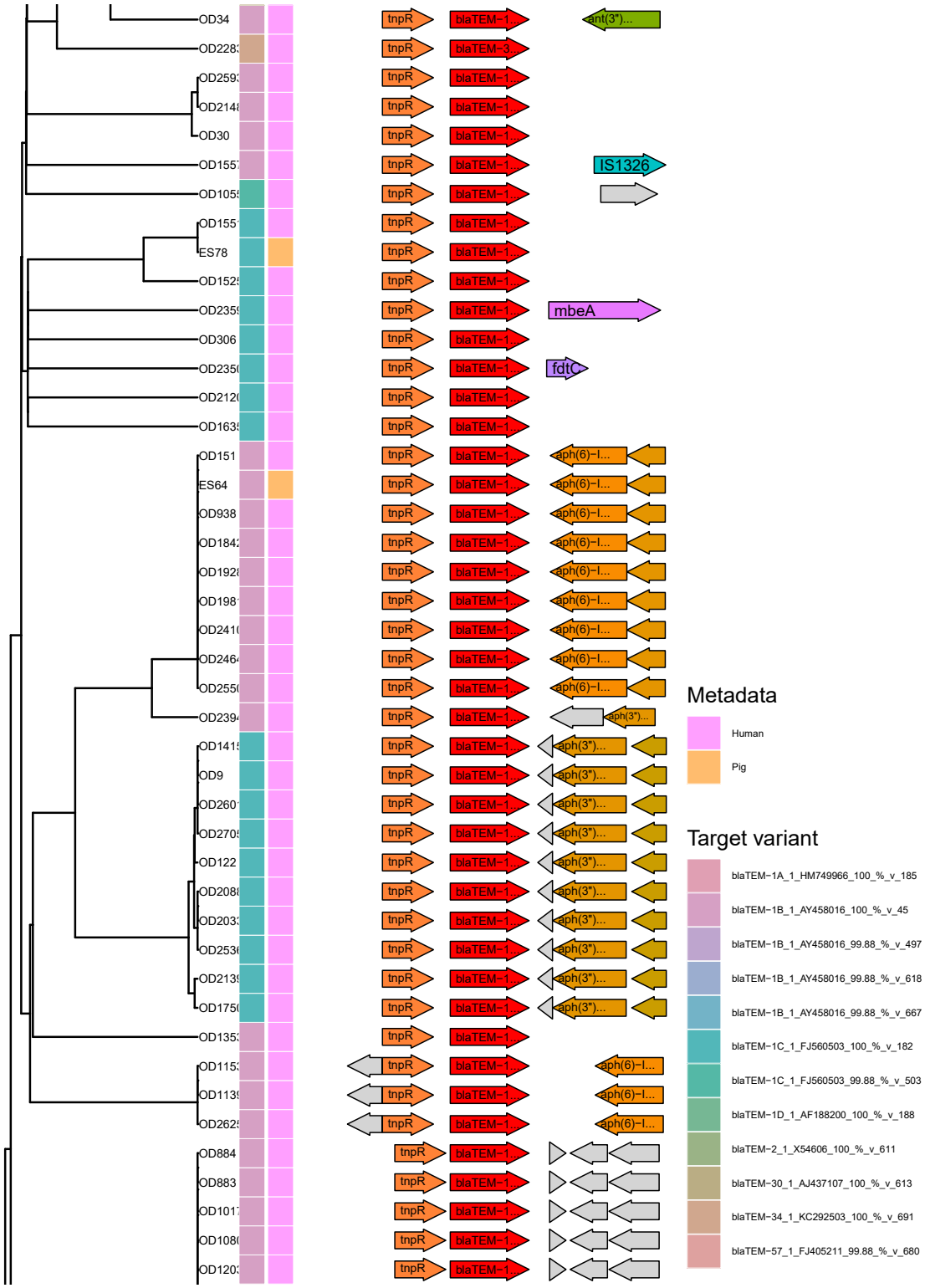
References

[1] Flankophile is available at https://genomicepidemiology.org/services/

continued ... Textbox 3.1

Figure 1 Two β-lactamase antimicrobial resistance genes from the blaTEM family of pig origin (orange) were found to be identical in both gene and flanking region sequences to those of human origin (pink) DANMAP 2022



Example of an output of Flankophile. Detail of a Flankophile plot from the study of a collection of bacterial samples from pigs and humans from Denmark (the plot has been cropped due to size). It is a gene synteny plot of all blaTEM gene hits from the study. From left to right: 1) Distance tree of the blaTEM flanking regions (straight vertical lines indicate that the flanking regions are 100% identical); 2) Color annotation columns representing the target variant (left) and the host species (right); 3) Arrows depicting the gene synteny, with the target sequence in the middle (red)