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. Moreover, integration happens when discussing resistance findings in indicator and pathogenic bacteria and using it as a basis for recommendations and treatment guidelines among different participants of the programme. Finally, DANMAP supports the development and definition of strategies and action plans to reduce AMR 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 possible relationship between the veterinary, food-producing and human sectors concerning antimicrobial usage (AMU) and development of antimicrobial resistance (AMR). To be able to foresee if changes in one sector will have a possible significant impact on the other sector, it requires knowledge of the probable 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 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 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 cross-analyse antimicrobial resistance data from monitoring in livestock animals and humans in Denmark. 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. Furthermore, we study the genomic context of selected resistance genes detected in those isolates, which may have been horizontally transferred between reservoirs. 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.

The annual number of bloodstream infections in humans in Denmark caused by ESBL Ec has been decreasing since 2019 (see Chapter 8, section 8.2.1). Similarly, a significant reduction in ESBL Ec has been observed in Danish broilers (DANMAP 2022, Chapter 7, section 7.3.1), cattle and pigs (see Chapter 7, section 7.3.1), as well as among domestic and imported meat (DANMAP 2022, 2023, Chapter 7, section 7.3.1). In this chapter, we demonstrate, as previously done, possible relationships between ESBL Ec from different sources in Denmark, and we furthermore deepen the genomic analyses to further investigate the possible occurrence of zoonotic transmission.

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

3.2.1 Abundance distribution of MLSTs and ESBL/AmpC genotypes

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 2023; 91 isolates of animal origin and 225 isolates from humans, totaling a dataset of 1,964 ESBL isolates from humans and animals from 2018 through 2023. The 1,282 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: 90, broiler meat: 145, cattle: 56, beef: 41, pigs: 219, pork: 50, and turkey meat: 81) stem from the EU mandatory screening programme from healthy animals and meat products (see Chapter 10 for more information).

Each isolate had 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 2023 in *E. coli* from bloodstream infections, and from cattle, pigs, beef and pork, see Chapter 8 (Tables 8.15 and 8.16), 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.

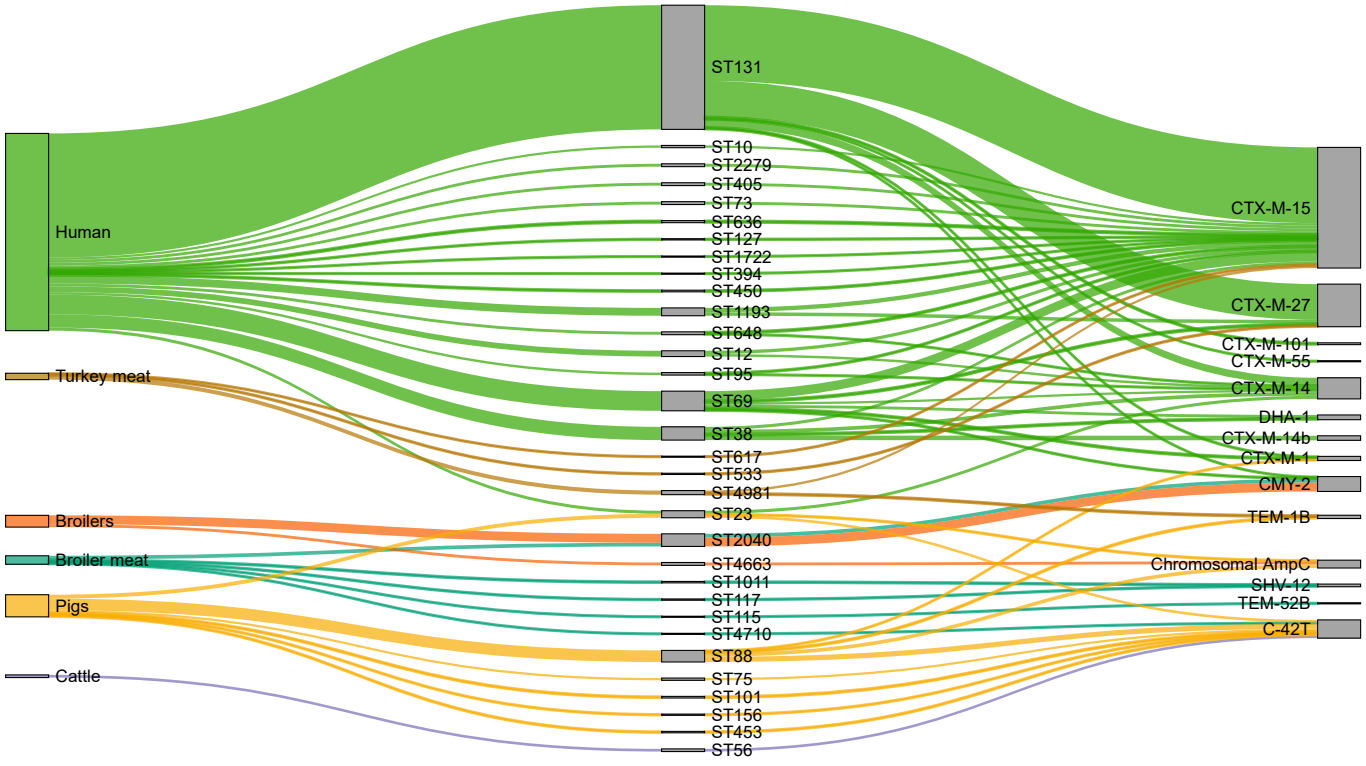
As in the previous years, limited overlap was found in both STs and ESBL/AmpC genes and mutations in isolates from humans vs. animals and food (Figure 3.1).

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 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*.

Only the AmpC plasmid-mediated gene *CMY-2*, and the ESBL genes *CTX-M-1*, *CTX-M-15* and *CTX-M-27* were found in both humans and food-producing animals or meat. 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), 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. The *CTX-M-1* gene was mostly found among human isolates, but also in pig isolates of ST88. All isolates from cattle harboured a C-42T mutation, which was not detected among the selected human isolates. Notably, isolates from broilers were of two types; ST2040 carrying a *CMY-2* gene or ST4663 harbouring a chromossomal AmpC mutation.

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 five years, and when considering the occurrence of ESBL/ AmpC genotypes in different sequence types. Further research into slow transmission over longer time spans, as well as more in depth genomic analyses could be of interest.

Figure 3.1 A Sankey diagram comprised of 1202 ESBL Ec isolates from humans, animals and food showing the relationship between the isolates' source, sequence type and ESBL/AmpC gene or mutation DANMAP 2023



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

3.2.2 Genomic context of ESLB/AmpC genes

As described above and in DANMAP 2022 (Chapter 3, Textbox 3.1), it is difficult to establish a clearly distinct transmission link for most ESBL genes between animals, meat and humans. Rarely the same acquired antimicrobial resistance genes (ARGs) are detected in different reservoirs, but for these, the analysis conclusion of the DNA sequence of the ARGs flanking region, i.e. the genetic code that comes before and after a gene, can assist in the determination of a common ARG source.

Here we applied the bioinformatics tool Flankophile [http://www.genomicepidemiology.org/services], for analysis and visualization of flanking region synteny of selected ESBL/AmpC genes, to a selected dataset of 1,557 sequenced ESBL Ec isolates from humans, animals and meat, gathered in the period 2018-2023 (see section 3.1 above).

We focused the analysis on resistance genes also present in figure 3.1, i.e. those identified in at least five isolates from each combination of source and MLST. This included the ESBL genes *CTX-M-1*, *CTX-M-14*, *CTX-M-15*, *CTX-M-27*, *CTX-M-55*, *TEM-52B*, and the AmpC genes *DHA-1* and *CMY-2*. Considering hierarchical clustering of variants according to both resistance gene and flanking region sequences, the results showed the following:

CTX-M-1: two variants were identified exclusively in broiler meat and broilers; three variants where present in both broilers or broiler meat, and pigs or pork; two variants were identified in pigs and humans, with one of those being also present in turkey meat.

CTX-M-14: most variants were exclusively identified in humans; one variant was identified in humans and broilers, broiler meat, pigs and duck meat.

CTX-M-15: several variants were present in both humans and an animal host, including three clusters with pigs, cattle, and turkey meat; two clusters showed the presence of the same variant in turkey meat and in beef and cattle.

CTX-M-27: there were no identical variants identified among human- and animal isolates; all variants were identified in humans, except for a single cluster observed in turkey meat.

CTX-M-55: two variants were identified in pork and humans; one cluster included turkey meat and broiler meat.

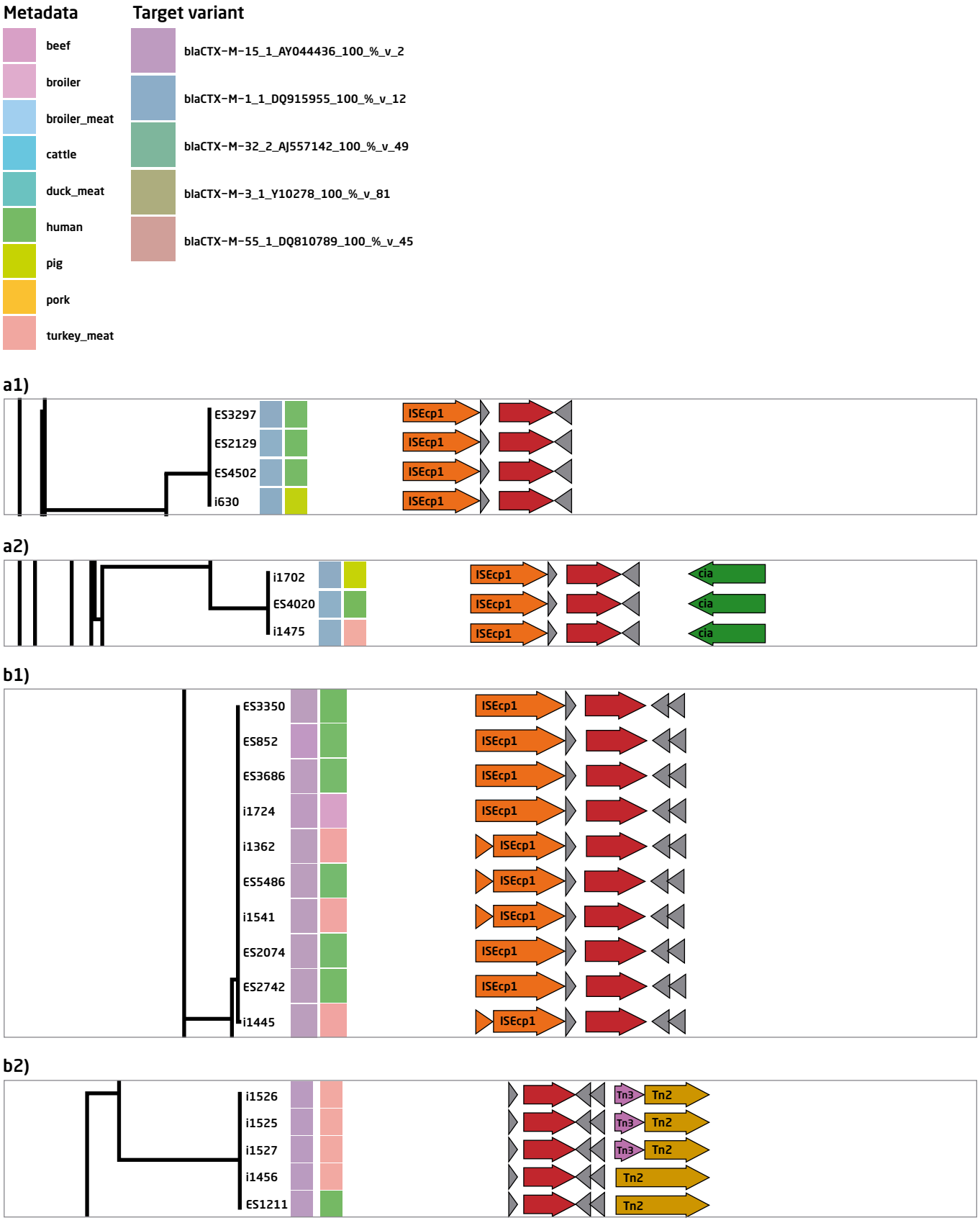
TEM-52B: there were no identical variants identified among human- and animal isolates.

DHA-1: two variants were identified in both humans and animal hosts, one in pigs only, and another in broilers, cattle, pigs and pork.

CMY-2: there were no identical variants identified among human- and animal isolates; one variant was identified in broilers, pork and beef.

In sum, among those ARGs included in the analysis, the comparison of the gene sequence and its flanking region showed some examples where the gene variant and its entire 2,500 base pair flanking region was identical in isolates from humans and from one or more animal hosts. There were also examples where no such overlap was observed. Furthermore, there were cases of overlap between different animal hosts, but not with humans.

Figure 3.2 ESBL genes *CTX-M-1* (a1, a2) and *CTX-M-15* (b1, b2) identical in both gene and flanking region sequences between *E. coli* isolates of animal origin and human origin DANMAP 2023



Details from Flankophile plots, showing a1 – clustering of *CTX-M-1* genes of human- and pig origin; a2 - clustering of *CTX-M-1* genes of human-, pig- and turkey meat origin; b1 - clustering of *CTX-M-15* genes of human-, turkey meat and beef origin; b2 - clustering of *CTX-M-15* genes of human- and turkey meat origin. From left to right: distance tree of the gene's flanking regions (straight vertical lines indicate that the flanking regions are 95% identical); color annotation columns representing the target variant (left) and the host species (right); arrows depicting the gene synteny, with the target sequence in red

3.3 Conclusion and future perspectives

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

The comparison of ESBL/AmpC-producing *E. coli* isolates from livestock animals, meat and human bloodstream infections suggests, as previously, limited overlap between the sources with regards to the combination of sequence type and ESBL/AmpC-genes.

The further analysis of the genomic background of the ESBL/AmpC-genes, including the gene sequence and the flanking regions, contributed to further investigate the potential common origin of the genes, regardless of the sequence type of the isolates. The results of both analyses are in accordance regarding *CTX-M-1* being possibly shared between isolates from pigs and humans, as well as *CTX-M-15* being possibly shared between humans and turkey meat. The flanking region analysis clarified that the *CTX-M-27* present in turkey meat isolates, as well as the *CMY-2* present in isolates from broilers and broiler meat do not overlap with the variant present in human isolates.

While the comparison of sequence type and ESBL/AmpC genes combinations shows little to no overlap between ESBL Ec of different hosts, the gene sequence and flanking region analysis indicates a probable transmission of ESBL/AmpC genes across *E. coli* from different hosts via horizontal transfer. The zoonotic transmission of ESBL/AmpC-producing *E. coli* thus warrant continued monitoring and further studies, to provide a deeper comprehension of transmission between sectors.

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