# 9

# RESISTANCE IN ANIMAL PATHOGENS

# 9. Resistance in animal pathogens

## Highlights

Surveillance of antimicrobial resistance in 2022 focused on pathogenic bacteria from pigs and included results obtained through antimicrobial susceptibility (AST) testing and/or whole genome sequencing (WGS) of isolates belonging to *Actinobacillus pleuropneumoniae* (AST and WGS), *Bordetella bronchiseptica* (AST and WGS), *Clostridium perfringens* (WGS), *Erysipelothrix rhusiopathiae* (WGS), haemolytic and non-haemolytic *Escherichia coli* (AST and WGS), *Glaesserella parasuis* (WGS), *Klebsiella pneumoniae* (AST and WGS), *Salmonella enterica* (AST and WGS), *Staphylococcus hyicus* (AST and WGS) and *Streptococcus suis* (AST and WGS).

AST showed that most pathogenic bacteria from pigs displayed similar frequencies of phenotypic resistance as in previous years.

A notable exception was the increased frequency of neomycin resistance in haemolytic *E. coli*, from 6.9% in 2016 to 43.2% in 2022. This is concerning because it is one of only a few drugs recommended in Denmark as first choice for treating *E. coli*-associated post-weaning diarrhoea. The rapid increase in neomycin resistance might, at least in part, be due to increased use of neomycin in weaners.

WGS demonstrated that resistance towards antimicrobial agents considered critically important for human medicine remained at a low level.

The observed concordance between AST results and WGS-based detection of resistance genes and point mutations was 99.7% for *A. pleuropneumoniae*, 64.5% for *B. bronchiseptica*, 92.8% for haemolytic *E. coli*, 93.9% for non-haemolytic *E. coli*, 61.7% for *K. pneumoniae*, 95.7% for *S. enterica*, 92.6% for *S. hyicus* and 94.0% for *S. suis*.

## 9.1 Introduction

Antimicrobial susceptibility testing (AST) and surveillance of antimicrobial resistance (AMR) in pathogenic bacteria from pigs, including *Actinobacillus pleuropneumoniae*, haemolytic *Escherichia coli* and *Streptococcus suis*, have been part of the DANMAP programme since 2015. In 2020, the Danish Veterinary and Food Administration (DVFA) asked the Danish Veterinary Consortium (DK-VET) to investigate whether it would be possible to implement whole genome sequencing (WGS) in the surveillance of AMR in pathogenic bacteria from food-producing animals as a basis to detect resistance genes and point mutations. WGS-based AMR surveillance in pathogenic bacteria from pigs commenced in January 2021 and included AST and/or WGS of isolates belonging to *A. pleuropneumoniae* (AST and WGS) *Bordetella bronchiseptica* (AST and WGS), *Clostridium perfringens* (WGS), *Erysipelothrix rhusiopathiae* (WGS), haemolytic and non-haemolytic *E. coli* (AST and WGS), *Glaesserella parasuis* (WGS), *Klebsiella pneumoniae* (AST and WGS), *Salmonella enterica* (AST and WGS), *Staphylococcus hyicus* (AST and WGS) and *S. suis* (AST and WGS), which were identified in clinical samples submitted by veterinarians to the Veterinary Laboratory, The Danish Agriculture and Food Council.

## 9.2 Temporal trends of AMR in pathogenic bacteria from pigs

The Veterinary Laboratory performed AST of isolates belonging to *A. pleuropneumoniae*, *B. bronchiseptica*, haemolytic and non-haemolytic *E. coli*, *K. pneumoniae*, *S. enterica*, *S. hyicus* and *S. suis*. Table 9.1 shows the frequencies of resistant isolates in 2022, while results from 2016-2021 can be found on DK-VET's homepage (https://www.vetssi.dk/). Data are based on epidemiological cut-offs (ECOFFs) and clinical breakpoints when ECOFFs are unavailable (https://www.vetssi.dk/).

**Table 9.1 Phenotypic antimicrobial resistance among pathogenic bacteria from pigs, Denmark, 2022** DANMAP 2022

| Antimicrobial agent | Ap | Bb | H-Ec | NH-Ec | Kp | Se | Sh | Ss |
|---|---|---|---|---|---|---|---|---|
| | R (%) | R (%) | R (%) | R (%) | R (%) | R (%) | R (%) | R (%) |
| Amoxicillin | 0.0% | ND | 70.8% | 85.6% | ND | 68.6% | 75.0% | ND |
| Amoxicillin/clavulanic acid | ND | ND | 13.5% | 18.0% | 5.6%* | 7.1% | ND | ND |
| Ampicillin | 0.0% | 100.0%* | 60.9% | 71.4% | 100.0%* | 85.7% | ND | ND |
| Cefotaxime | ND | ND | 4.3% | 2.9% | 0.0%* | 0.0% | ND | ND |
| Cefpodoxime | ND | ND | 0.0% | 0.0% | ND | ND | ND | ND |
| Cefquinome | ND | ND | ND | ND | 0.0%* | ND | ND | ND |
| Ceftiofur | 1.1% | ND | 0.0% | 0.0% | 0.0%* | 0.0% | ND | ND |
| Chloramphenicol | ND | ND | 21.7% | 22.9% | 50.0%* | 42.9% | ND | 1.1% |
| Ciprofloxacin | ND | ND | 13.0% | 0.0% | 0.0%* | 0.0% | ND | ND |
| Colistin | ND | ND | 1.0% | 0.0% | 0.0%* | ND | ND | ND |
| Doxycycline | 0.0% | ND | 51.6% | 51.0% | 28.6%* | 74.3% | ND | 39.0% |
| Enrofloxacin | 3.4% | ND | 9.2% | 3.8% | ND | ND | 0.0%* | 0.0% |
| Erythromycin | 0.0% | ND | ND | ND | ND | ND | ND | 68.9% |
| Florfenicol | 0.0% | 0.0% | 16.9% | 16.5% | 11.1%* | 26.2% | 0.0%* | 1.2% |
| Gentamicin | ND | ND | 19.3% | 13.0% | 0.0%* | 19.0% | ND | ND |
| Lincomycin | ND | ND | ND | ND | ND | ND | 100.0%* | ND |
| Nalidixic acid | ND | ND | 4.3% | 0.0% | ND | 0.0% | ND | ND |
| Neomycin | ND | ND | 43.2% | 26.1% | ND | 28.6% | ND | ND |
| Penicillin | 1.1% | ND | ND | ND | ND | ND | 75.0%* | 0.6% |
| Spectinomycin | ND | ND | 57.4% | 44.9% | ND | ND | ND | ND |
| Streptomycin | ND | ND | 78.0% | 82.0% | ND | 81.0% | ND | ND |
| Tetracycline | 3.4% | ND | 73.3% | 70.3% | 27.8%* | 76.2% | ND | 35.6% |
| Tiamulin | 0.0% | ND | ND | ND | ND | ND | 100.0%* | ND |
| Tildipirosin | 0.0% | 0.0%* | ND | ND | ND | ND | ND | ND |
| Tilmicosin | 0.0% | ND | ND | ND | ND | ND | 0.0%* | ND |
| Trimethoprim | ND | ND | 58.7% | 65.7% | 50.0%* | 71.4% | ND | ND |
| Trimethoprim/sulfamethoxazole | 0.0% | ND | 54.8% | 72.1% | 42.9%* | 45.7% | 50.0%* | 16.3% |
| Tulathromycin | 0.0% | 3.3% | ND | ND | ND | ND | ND | ND |
| Tylosin | ND | ND | ND | ND | ND | ND | 0.0%* | ND |

Data are based on epidemiological cut-offs (ECOFFs) and clinical breakpoints when ECOFFs are unavailable (https://www.vetssi.dk/)
Percentages based on small sample sizes (n<20) are indicated by asterices and should be interpreted with caution
Abbreviations: Ap, *Actinobacillus pleuropneumoniae*; Bb, *Bordetella bronchiseptica*; H-Ec, haemolytic *Escherichia coli*; NH-Ec, non-haemolytic *Escherichia coli*; Kp, *Klebsiella pneumoniae*; Se, *Salmonella enterica*; Sh, *Staphylococcus hyicus*; Ss, *Streptococcus suis,* R, resistant; ND, not determined

Most pathogenic bacteria from pigs displayed similar frequencies of phenotypic resistance as in previous years. Table 9.2 and Figure 9.1 show all significant changes in phenotypic resistance over a 1-year period (2022 vs. 2021) or a 5-year period (2022 vs. 2017).

Haemolytic *E. coli* displayed significantly increased resistance to florfenicol, gentamicin, neomycin and tetracycline (Figure 9.1). The high frequency of neomycin resistance in haemolytic *E. coli* (43.2%) is particularly worrisome because neomycin is one of only a few drugs recommended in Denmark as first choice for treating *E. coli*-associated post-weaning diarrhoea. Furthermore, haemolytic *E. coli* also displayed medium to high frequencies of resistance to the other first-choice drugs, including amoxicillin/clavulanic acid (13.5%), ampicillin (60.9%), spectinomycin (57.4%), trimethoprim/sulfamethoxazole (54.8%) and streptomycin (78.0%). The rapid increase in neomycin resistance might, at least in part, be due to increased use of neomycin in weaners (Figure 9.2) following two recent decisions to restrict the use of alternative drugs in pigs: 1) the Danish Yellow Card initiative to reduce the use of colistin in 2016 and 2) the European Union-wide ban of medicinal zinc in 2022. It should be noted that we also observed a significant increase in neomycin resistance in non-haemolytic *E. coli* (Figure 9.1). *S. enterica* displayed significantly increased resistance to florfenicol and trimethoprim, while *S. suis* displayed significantly increased resistance to erythromycin (Figure 9.1).

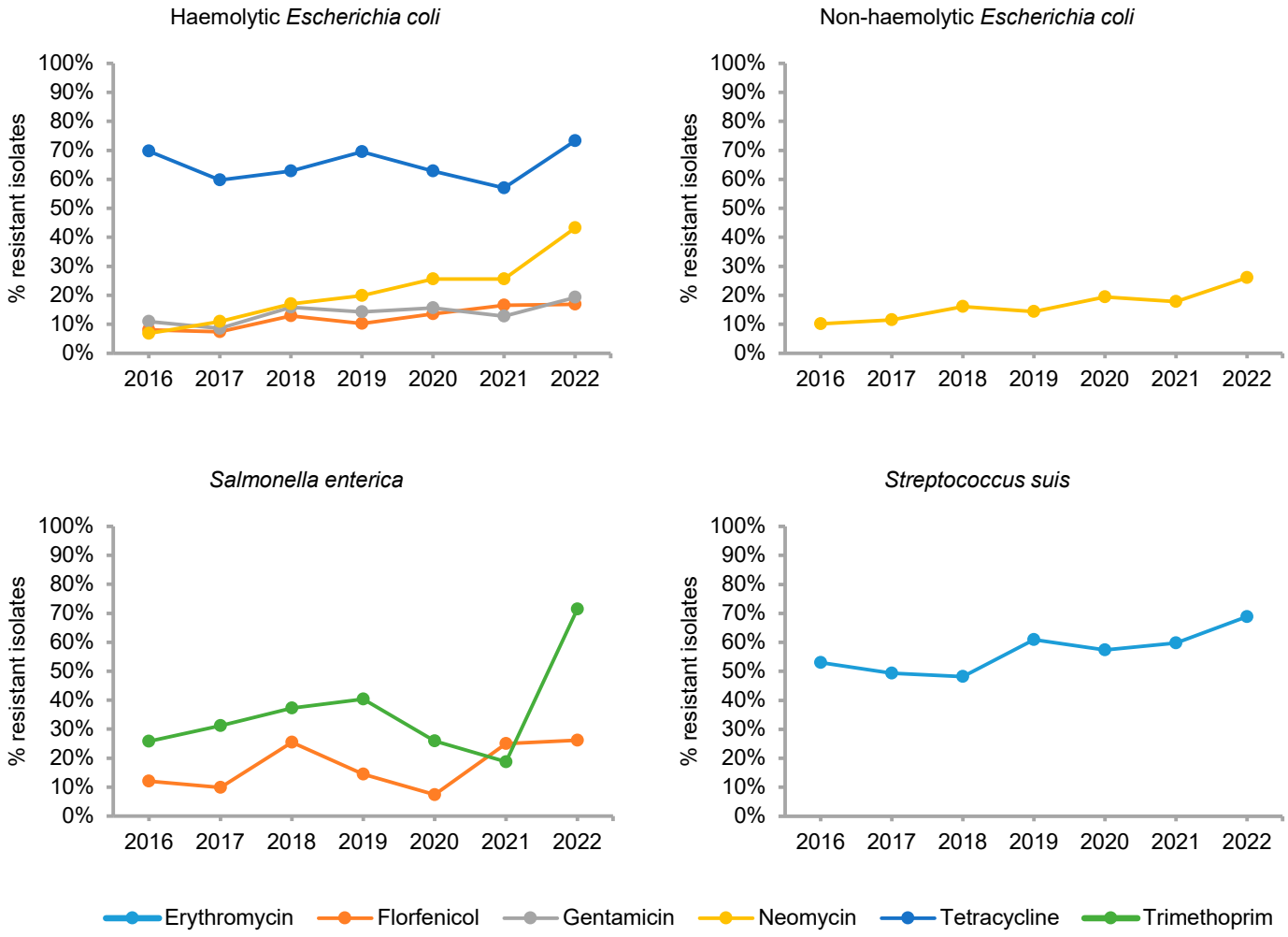
**Table 9.2 Temporal changes in antimicrobial resistance phenotypes among pathogenic bacteria from pigs, Denmark, 2021-2022 and 2017-2022**

DANMAP 2022

| Pathogen | Antimicrobial agent | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2022 vs. 2021 | 2022 vs. 2017 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | R (%) | R (%) | R (%) | R (%) | R (%) | R (%) | R (%) | P value | P value |
| H-Ec | Florfenicol | 8.1% | 7.4% | 12.9% | 10.3% | 13.6% | 16.5% | 16.9% | 1.0000 | 0.0008 |
| H-Ec | Gentamicin | 10.9% | 8.6% | 15.9% | 14.3% | 15.6% | 12.8% | 19.3% | 0.0470 | 0.0004 |
| H-Ec | Neomycin | 6.9% | 10.9% | 17.0% | 19.8% | 25.6% | 25.6% | 43.2% | 0.0000 | 0.0000 |
| H-Ec | Tetracycline | 69.8% | 59.8% | 62.9% | 69.4% | 62.8% | 57.0% | 73.3% | 0.0001 | 0.0008 |
| NH-Ec | Neomycin | 10.2% | 11.5% | 16.1% | 14.4% | 19.4% | 17.8% | 26.1% | 0.0638 | 0.0008 |
| Se | Florfenicol | 12.1% | 9.8% | 25.5% | 14.5% | 7.4% | 25.0% | 26.2% | 1.0000 | 0.0338 |
| Se | Trimethoprim | 25.9% | 31.1% | 37.3% | 40.3% | 25.9% | 18.8% | 71.4%* | 0.0122 | 0.0875 |
| Ss | Erythromycin | 53.0% | 49.3% | 48.2% | 61.0% | 57.3% | 59.7% | 68.9% | 0.1684 | 0.0032 |

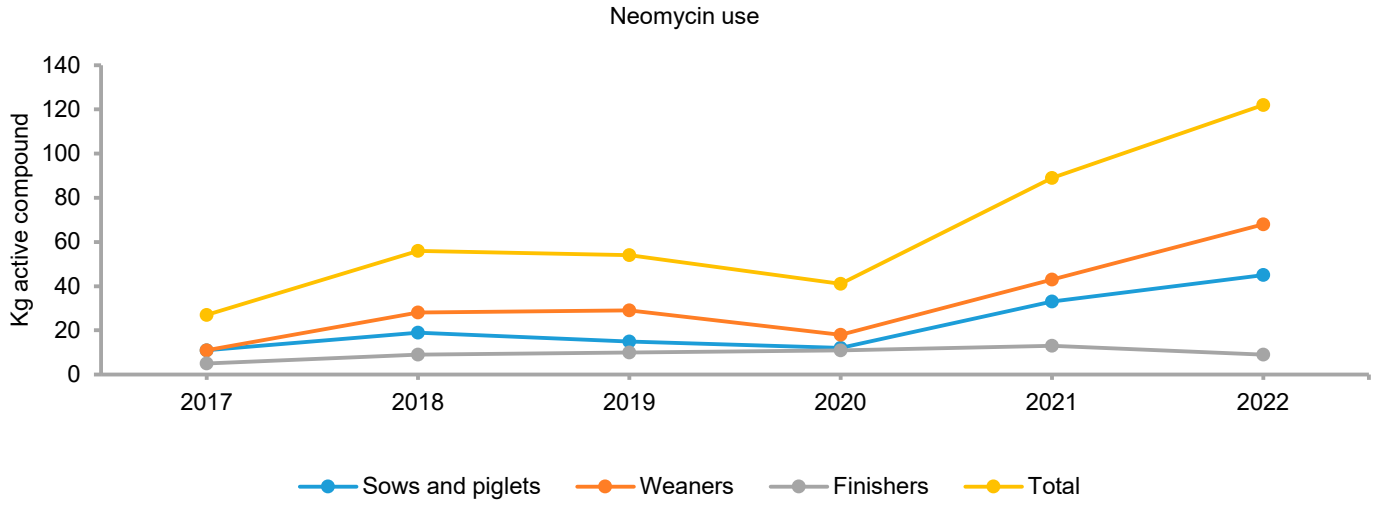
Antimicrobial resistance phenotypes that remained at the same level during 2021-2022 and 2017-2022 were excluded (https://www.vetssi.dk/)
Percentages based on small sample sizes (n<20) are indicated by asterices and should be interpreted with caution
Abbreviations: H-Ec, haemolytic *Escherichia coli*; NH-Ec, non-haemolytic *Escherichia coli*; Se, *Salmonella enterica*; Ss, *Streptococcus suis;* R, resistant

**Figure 9.1 Phenotypic antimicrobial resistance among pathogenic bacteria from pigs, Denmark, 2016-2022** DANMAP 2022




Antimicrobial resistance phenotypes that remained at the same level during 2021-2022 and 2017-2022 were excluded (https://www.vetssi.dk/)
The percentages of trimethoprim-resistant *Salmonella enterica* isolates are based on small sample sizes (n<20) and should therefore be interpreted with caution

**Figure 9.2 Neomycin use in the total pig population and in each age group, Denmark 2017-2022** DANMAP 2022



## 9.3 WGS-based detection of resistance genes and point mutations

WGS of a randomly selected subset of *A. pleuropneumoniae* (n=218), *B. bronchiseptica* (n=54), *C. perfringens* (n=140), *E. rhusiopathiae* (n=2), haemolytic *E. coli* (n=214), non-haemolytic *E. coli* (n=144), *G. parasuis* (n=90), *K. pneumoniae* (n=28), S. enterica (n=58), *S. hyicus* (n=11) and *S. suis* (n=250) isolates from 2021 and 2022 was subjected to WGS. Table 9.3 provides a list of the detected resistance genes and point mutations in *A. pleuropneumoniae*, haemolytic *E. coli* and *S. suis* from both 2021 and 2022. A full list of resistance genes and point mutations detected in isolates from both 2021 and 2022 can be found on DK-VET's homepage (https://www.vetssi.dk/).

Of note, *aph(3')-Ia* encoding resistance to neomycin was present in 33.9% and 31.4% of the haemolytic *E. coli* isolates and in 21.2% and 17.4% of the non-haemolytic *E. coli* isolates from 2022 and 2021, respectively. No other neomycin resistance genes were detected in *E. coli*. Interestingly, *aph(3')-Ia* was also present in 12.5% of the *K. pneumoniae* isolates from 2022 and in 28.2% and 21.1% of the *S. enterica* isolates from 2022 and 2021, respectively, while it was absent in *K. pneumoniae* isolates from 2021. In addition, *aph(3')-Ia* was present in 2.0% of the *G. parasuis* isolates from 2021 but absent in *G. parasuis* isolates from 2022. These observations suggest that *aph(3')-Ia* is present on a mobile genetic element that can be horizontally transferred within and between different species. Most of the susceptibility tested *aph(3')-Ia*-positive *E. coli* and *S. enterica* isolates were phenotypically resistant to neomycin, except from two out of 68 haemolytic *E. coli* isolates, one out of 26 non-haemolytic *E. coli* isolates and one out of 14 *S. enterica* isolates. *K. pneumoniae* and *G. parasuis* isolates were not tested for susceptibility to neomycin, and their phenotype is therefore unknown.

Some isolates harboured genes/point mutations associated with resistance towards antimicrobial agents considered critically important for human medicine by the World Health Organization. Here we focus on genes and mutations that confer resistance to carbapenems, 3rd, 4th and 5th generation cephalosporins (e.g., the 3rd generation cephalosporin cefotaxime), oxazolidinones (e.g., linezolid) and polymyxins (e.g., colistin).

For *C. perfringens*, *cfr*(B) and *cfr*(E) associated with resistance to linezolid were present in 1.8% and 3.6% of the isolates from 2022 but absent in isolates from 2021, while *cfr*(C) was present in 8.3% of the isolates from 2021 but absent in isolates from 2022. *cfr* genes are usually plasmid-borne and confer transferable resistance not only to linezolid and other oxazolidinone but also to lincosamides, phenicols, pleuromutilins and streptogramins, and it is therefore possible that use of other antimicrobial agents can co-select for linezolid resistance.

*optrA*, another gene associated with transferable resistance to linezolid and other oxazolidinone as well as to phenicols, was present in 1.4% and 1.9% of the *S. suis* isolates from 2022 and 2021, respectively. *C. perfringens* and *S. suis* isolates were not tested for susceptibility to linezolid, and their phenotype is therefore unknown.

The extended-spectrum β-lactamase (ESBL)-encoding $bla_{TEM-169}$ gene was present in 1.9% of the non-haemolytic *E. coli* isolates from 2022, while it was absent in non-haemolytic *E. coli* isolates from 2021. The ESBL-encoding $bla_{CTX-M-1}$ gene was present in 1.0% of the haemolytic *E. coli* isolates from 2021 but absent in haemolytic *E. coli* isolates from 2022. In addition, we detected two distinct extended-spectrum β-lactam resistance-associated mutations in the *ampC* promoter, a -32T→A transversion and a -42C→T transition, in 2.8% and 0.9% of the haemolytic *E. coli* isolates from 2022, compared with 1.9% and 3.8% of the haemolytic *E. coli* isolates from 2021. Seven out of eight susceptibility tested haemolytic *E. coli* isolates harbouring $bla_{CTX-M-1}$ and/or point mutations in the *ampC* promoter were phenotypically resistant to cefotaxime, with the only exception being a single haemolytic *E. coli* isolate harbouring a -32T→A transversion in the *ampC* promoter. The single non-haemolytic *E. coli* isolate harbouring $bla_{TEM-169}$ was not susceptibility tested, and its phenotype is therefore unknown. $bla_{SHV-27}$, $bla_{SHV-110}$ and $bla_{SHV-185}$ were detected in 6.3%, 87.5% and 6.3% of the *K. pneumoniae* isolates from 2022, compared with 0.0%, 100.0% and 0.0% of the *K. pneumoniae* isolates from 2021. These genes are considered to be naturally occurring in *K. pneumoniae*, where they encode an ESBL, a broad-spectrum β-lactamase and a hitherto uncharacterised β-lactamase, respectively (http://bldb.eu/). In addition, all *K. pneumoniae* isolates from both 2022 and 2021 harboured point mutations in *ompK36* and/or *ompK37* associated with resistance to cephalosporins. However, none of the 15 susceptibility tested *K. pneumoniae* isolates harbouring $bla_{SHV}$ genes and/or point mutations in *ompK36* and *ompK37* were phenotypically resistant to cefotaxime.

We did not detect any genes encoding resistance to carbapenems or colistin, although it should be noted that we identified a point mutation in *pmrB* associated with resistance to colistin in 14.7% and 14.3% of the haemolytic *E. coli* isolates and 1.9% and 3.3% of the non-haemolytic *E. coli* isolates from 2022 and 2021, respectively. However, only two out of 35 susceptibility tested *E. coli* isolates harbouring the point mutation in *pmrB* were phenotypically resistant to colistin. All *K. pneumoniae* isolates harboured point mutations in *ompK36* and/or *ompK37* associated with resistance to carbapenems, but their phenotype is unknown as they were not tested for susceptibility to this antimicrobial subclass.

**Table 9.3 Antimicrobial resistance genes and mutations identified through whole genome sequencing of pathogenic bacteria from pigs, Denmark, 2021-2022** DANMAP 2022

| Pathogen | Resistance gene/mutation | Class | Phenotype | 2021 | 2022 | 2022 vs. 2021 |
|---|---|---|---|---|---|---|
| | | | | % | % | P value |
| Ap | *aph(3'')-Ib* | Aminoglycoside | Streptomycin | 0.0% | 1.1% | 0.4128 |
| | *bla*$_{ROB-1}$ | β-lactam | Penicillin, amoxicillin, ampicillin | 0.8% | 1.1% | 1.0000 |
| | *sul2* | Folate pathway antagonist | Sulfamethoxazole | 0.0% | 1.1% | 0.4128 |
| | *tet*(B) | Tetracycline | Doxycycline, tetracycline, minocycline | 3.1% | 3.3% | 1.0000 |
| H-Ec | *aac(3)-IId* | Aminoglycoside | Apramycin, gentamicin, tobramycin, dibekacin, netilmicin, sisomicin | 1.9% | 0.9% | 0.6163 |
| | *aac(3)-IV* | Aminoglycoside | Gentamicin, tobramycin | 9.5% | 22.0% | 0.0148 |
| | *aadA1* | Aminoglycoside | Spectinomycin, streptomycin | 41.0% | 44.0% | 0.6798 |
| | *aadA2* | Aminoglycoside | Spectinomycin, streptomycin | 17.1% | 11.0% | 0.2387 |
| | *aadA3* | Aminoglycoside | Spectinomycin, streptomycin | 2.9% | 4.6% | 0.7217 |
| | *aadA4* | Aminoglycoside | Spectinomycin, streptomycin | 0.0% | 1.8% | 0.4978 |
| | *aadA5* | Aminoglycoside | Spectinomycin, streptomycin | 1.0% | 3.7% | 0.3695 |
| | *aadA7* | Aminoglycoside | Spectinomycin, streptomycin | 0.0% | 0.9% | 1.0000 |
| | *aadA11* | Aminoglycoside | Spectinomycin, streptomycin | 1.0% | 1.8% | 1.0000 |
| | *aadA12* | Aminoglycoside | Spectinomycin, streptomycin | 17.1% | 11.9% | 0.3330 |
| | *aadA13* | Aminoglycoside | Spectinomycin, streptomycin | 1.9% | 3.7% | 0.6834 |
| | *aadA17* | Aminoglycoside | Spectinomycin, streptomycin | 0.0% | 3.7% | 0.1217 |
| | *aadA22* | Aminoglycoside | Spectinomycin, streptomycin | 1.0% | 0.9% | 1.0000 |
| | *ant(2'')-Ia* | Aminoglycoside | Gentamicin, tobramycin | 1.0% | 1.8% | 1.0000 |
| | *ant(3'')-Ia* | Aminoglycoside | Streptomycin | 26.7% | 30.3% | 0.6499 |
| | *aph(3'')-Ib* | Aminoglycoside | Streptomycin | 46.7% | 57.8% | 0.1320 |
| | *aph(3')-Ia* | Aminoglycoside | Neomycin, kanamycin, lividomycin, paromomycin, ribostamycin | 31.4% | 33.9% | 0.7711 |
| | *aph(4)-Ia* | Aminoglycoside | Hygromycin | 9.5% | 20.2% | 0.0349 |
| | *aph(6)-Id* | Aminoglycoside | Streptomycin | 44.8% | 51.4% | 0.3420 |
| | *bla*$_{CTX-M-1}$ | β-lactam | Amoxicillin, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftriaxone, piperacillin, ticarcillin | 1.0% | 0.0% | 0.4907 |
| | *bla*$_{TEM-1A}$ | β-lactam | Amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin | 1.0% | 4.6% | 0.2126 |
| | *bla*$_{TEM-1B}$ | β-lactam | Amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin | 61.9% | 63.3% | 0.8880 |
| | *bla*$_{TEM-1C}$ | β-lactam | Amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin | 1.0% | 1.8% | 1.0000 |
| | *bla*$_{TEM-30}$ | β-lactam | Amoxicillin, amoxicillin/clavulanic acid, ampicillin, ampicillin/clavulanic acid, piperacillin,piperacillin/ tazobactam, ticarcillin, ticarcillin/clavulanic acid | 1.9% | 2.8% | 1.0000 |
| | *bla*$_{TEM-127}$ | β-lactam | Amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin | 0.0% | 0.9% | 1.0000 |
| | *bla*$_{TEM-176}$ | β-lactam | Unknown β-lactam | 0.0% | 1.8% | 0.4978 |
| | *bleO* | Glycopeptide | Bleomycin | 1.0% | 0.9% | 1.0000 |
| | *catA1* | Amphenicol | Chloramphenicol | 1.9% | 3.7% | 0.6834 |
| | *cmlA1* | Amphenicol | Chloramphenicol | 15.2% | 6.4% | 0.0468 |
| | *dfrA1* | Folate pathway antagonist | Trimethoprim | 31.4% | 43.1% | 0.0904 |
| | *dfrA5* | Folate pathway antagonist | Trimethoprim | 5.7% | 2.8% | 0.3255 |
| | *dfrA8* | Folate pathway antagonist | Trimethoprim | 0.0% | 1.8% | 0.4978 |
| | *dfrA10* | Folate pathway antagonist | Trimethoprim | 0.0% | 0.9% | 1.0000 |
| | *dfrA12* | Folate pathway antagonist | Trimethoprim | 8.6% | 4.6% | 0.2780 |
| | *dfrA14* | Folate pathway antagonist | Trimethoprim | 5.7% | 2.8% | 0.3255 |
| | *dfrA16* | Folate pathway antagonist | Trimethoprim | 0.0% | 0.9% | 1.0000 |
| | *dfrA17* | Folate pathway antagonist | Trimethoprim | 1.0% | 2.8% | 0.6217 |
| | *dfrA32* | Folate pathway antagonist | Trimethoprim | 1.0% | 0.0% | 0.4907 |
| | *dfrA36* | Folate pathway antagonist | Trimethoprim | 1.0% | 0.9% | 1.0000 |
| | *ere*(A) | Macrolide | Erythromycin | 1.0% | 0.0% | 0.4907 |
| | *erm*(42) | Macrolide, lincosamide, streptogramin B | Erythromycin, lincomycin, clindamycin, quinupristin, pristinamycin IA, virginiamycin S | 0.0% | 1.8% | 0.4978 |

Abbreviations: Ap, *Actinobacillus pleuropneumoniae*; H-Ec, haemolytic *Escherichia coli*; Ss, *Streptococcus suis*

**continued ... Table 9.3 Antimicrobial resistance genes and mutations identified through whole genome sequencing of pathogenic bacteria from pigs, Denmark, 2021-2022** DANMAP 2022

| Pathogen | Resistance gene/mutation | Class | Phenotype | 2021 % | 2022 % | 2022 vs. 2021 P value |
|---|---|---|---|---|---|---|
| H-Ec | *erm*(B) | Macrolide, lincosamide, streptogramin B | Erythromycin, lincomycin, clindamycin, quinupristin, pristinamycin IA, virginiamycin S | 13.3% | 14.7% | 0.8452 |
| | *floR* | Amphenicol | Chloramphenicol, florfenicol | 16.2% | 17.4% | 0.8563 |
| | *lnu*(F) | Lincosamide | Lincomycin | 1.0% | 5.5% | 0.1193 |
| | *lnu*(G) | Lincosamide | Lincomycin | 2.9% | 4.6% | 0.7217 |
| | *mef*(B) | Macrolide | Erythromycin, azithromycin | 1.9% | 0.9% | 0.6163 |
| | *mph*(A) | Macrolide | Erythromycin, azithromycin, spiramycin, telithromycin | 15.2% | 19.3% | 0.4735 |
| | *mph*(B) | Macrolide | Erythromycin, spiramycin, telithromycin | 2.9% | 6.4% | 0.3329 |
| | *qnrS1* | Quinolone | Ciprofloxacin | 5.7% | 8.3% | 0.5948 |
| | *sul1* | Folate pathway antagonist | Sulfamethoxazole | 44.8% | 50.5% | 0.4151 |
| | *sul2* | Folate pathway antagonist | Sulfamethoxazole | 38.1% | 50.5% | 0.0750 |
| | *sul3* | Folate pathway antagonist | Sulfamethoxazole | 15.2% | 12.8% | 0.6952 |
| | *tet*(A) | Tetracycline | Doxycycline, tetracycline | 40.0% | 43.1% | 0.6787 |
| | *tet*(B) | Tetracycline | Doxycycline, tetracycline, minocycline | 21.0% | 35.8% | 0.0227 |
| | *tet*(C) | Tetracycline | Doxycycline, tetracycline | 1.0% | 0.0% | 0.4907 |
| | *tet*(G) | Tetracycline | Doxycycline, tetracycline | 1.0% | 0.0% | 0.4907 |
| | *tet*(M) | Tetracycline | Doxycycline, tetracycline, minocycline | 1.0% | 1.8% | 1.0000 |
| | *ampC* promoter T-32A | β-lactam | Ampicillin, ampicillin/clavulanic acid, amoxicillin, amoxicillin/clavulanic acid, cefixime, cefotaxime, cefoxitin, ceftazidime, piperacillin | 1.9% | 2.8% | 1.0000 |
| | *ampC* promoter C-42T | β-lactam | Ampicillin, ampicillin/clavulanic acid, amoxicillin, amoxicillin/clavulanic acid, cefixime, cefotaxime, cefoxitin, ceftazidime, piperacillin | 3.8% | 0.9% | 0.2058 |
| | *gyrA* S83L | Quinolone | Nalidixic acid, ciprofloxacin | 2.9% | 4.6% | 0.7217 |
| | *gyrA* D87Y | Quinolone | Nalidixic acid | 0.0% | 0.9% | 1.0000 |
| | *parC* F60I | Quinolone | Nalidixic acid, ciprofloxacin | 0.0% | 0.9% | 1.0000 |
| | *parC* S80I | Quinolone | Nalidixic acid, ciprofloxacin | 1.0% | 0.0% | 0.4907 |
| | *parC* S80R | Quinolone | Nalidixic acid, ciprofloxacin | 0.0% | 1.8% | 0.4978 |
| | *parC* E84K | Quinolone | Nalidixic acid, ciprofloxacin | 1.9% | 1.8% | 1.0000 |
| | *parE* I355T | Quinolone | Nalidixic acid, ciprofloxacin | 0.0% | 0.9% | 1.0000 |
| | *parE* I529L | Quinolone | Nalidixic acid, ciprofloxacin | 3.8% | 0.9% | 0.2058 |
| | pmrB V161G | Polymyxin | Colistin | 14.3% | 14.7% | 1.0000 |
| Ss | *ant(6)-Ia* | Aminoglycoside | Streptomycin | 19.4% | 19.0% | 1.0000 |
| | *ant(6)-Ib* | Aminoglycoside | Streptomycin | 0.9% | 0.7% | 1.0000 |
| | *aph(3')-III* | Aminoglycoside | Kanamycin, amikacin, neomycin, butirosin, isepamicin, lividomycin, paromomycin, ribostamycin | 7.4% | 7.0% | 1.0000 |
| | *erm*(47) | Macrolide, lincosamide, streptogramin B | Erythromycin, lincomycin, clindamycin, quinupristin, pristinamycin IA, virginiamycin S | 1.9% | 2.1% | 1.0000 |
| | *erm*(B) | Macrolide, lincosamide, streptogramin B | Erythromycin, lincomycin, clindamycin, quinupristin, pristinamycin IA, virginiamycin S | 57.4% | 64.8% | 0.2409 |
| | *lnu*(B) | Lincosamide | Lincomycin, clindamycin | 25.9% | 16.9% | 0.0862 |
| | *lnu*(C) | Lincosamide | Lincomycin | 0.0% | 0.7% | 1.0000 |
| | *lsa*(E) | Lincosamide, streptogramin A, pleuromutilin | Lincomycin, clindamycin, dalfopristin, pristinamycin IIA, virginiamycin M, tiamulin | 25.9% | 16.9% | 0.0862 |
| | *mef*(A) | Macrolide | Erythromycin, azithromycin | 4.6% | 2.1% | 0.2970 |
| | *msr*(D) | Macrolide, streptogramin B | Erythromycin, azithromycin, telithromycin, quinupristin, pristinamycin IA, virginiamycin S | 3.7% | 0.7% | 0.1689 |
| | *optrA* | Oxazolidinone, amphenicol | Linezolid, chloramphenicol, florfenicol | 1.9% | 1.4% | 1.0000 |
| | *tet*(40) | Tetracycline | Doxycycline, tetracycline | 0.0% | 0.7% | 1.0000 |
| | *tet*(M) | Tetracycline | Doxycycline, tetracycline, minocycline | 7.4% | 3.5% | 0.2497 |
| | *tet*(O) | Tetracycline | Doxycycline, tetracycline, minocycline | 30.6% | 16.9% | 0.0146 |

Abbreviations: Ap, *Actinobacillus pleuropneumoniae*; H-Ec, haemolytic *Escherichia coli*; Ss, *Streptococcus suis*

## 9.4 WGS-based prediction of AMR

WGS-based prediction of AMR was assessed by determining the concordance, sensitivity, specificity, positive predictive value, negative predictive value, major error rate and very major error rate between the results obtained through AST and WGS using the genotype-to-phenotype translations in the ResFinder 4.1 database. Table 9.4 shows the results for *A. pleuropneumoniae*, haemolytic *E. coli* and *S. suis* isolates from both 2021 and 2022, while results for all pathogen-drug combinations can be found on DK-VET's homepage (https://www.vetssi.dk/). The observed concordance was 99.7% for *A. pleuropneumoniae*, 64.5% for *B. bronchiseptica*, 92.8% for haemolytic *E. coli*, 93.9% for non-haemolytic *E. coli*, 61.7% for *K. pneumoniae*, 95.7% for *S. enterica*, 92.6% for *S. hyicus* and 94.0% for *S. suis*.

**Table 9.4 Diagnostic performance of ResFinder 4.1 as an antimicrobial resistance prediction tool for pathogenic bacteria from pigs, Denmark, 2021-2022** DANMAP 2022

| Pathogen | Antimicrobial agent | P+/G+ | P-/G- | G+/P- | G-/P+ | Concordance | Sensitivity | Specificity | PPV | NPV | ME rate | VME rate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ap | Amoxicillin | 0 | 28 | 0 | 0 | 100.0 | NA | 100.0 | NA | 100.0 | 0.0 | NA |
| | Ampicillin | 1 | 183 | 0 | 0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 0.0 | 0.0 |
| | Ceftiofur | 0 | 210 | 0 | 2 | 99.1 | 0.0 | 100.0 | NA | 99.1 | 0.0 | 100.0 |
| | Doxycycline | 0 | 27 | 1 | 0 | 96.4 | NA | 96.4 | 0.0 | 100.0 | 3.6 | NA |
| | Enrofloxacin | 0 | 27 | 0 | 1 | 96.4 | 0.0 | 100.0 | NA | 96.4 | 0.0 | 100.0 |
| | Erythromycin | 0 | 184 | 0 | 0 | 100.0 | NA | 100.0 | NA | 100.0 | 0.0 | NA |
| | Florfenicol | 0 | 212 | 0 | 0 | 100.0 | NA | 100.0 | NA | 100.0 | 0.0 | NA |
| | Penicillin | 1 | 209 | 0 | 2 | 99.1 | 33.3 | 100.0 | 100.0 | 99.1 | 0.0 | 66.7 |
| | Tetracycline | 6 | 178 | 0 | 0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 0.0 | 0.0 |
| | Tiamulin | 0 | 212 | 0 | 0 | 100.0 | NA | 100.0 | NA | 100.0 | 0.0 | NA |
| | Tildipirosin | 0 | 28 | 0 | 0 | 100.0 | NA | 100.0 | NA | 100.0 | 0.0 | NA |
| | Tilmicosin | 0 | 212 | 0 | 0 | 100.0 | NA | 100.0 | NA | 100.0 | 0.0 | NA |
| | Trimethoprim/ sulfamethoxazol | 0 | 212 | 0 | 0 | 100.0 | NA | 100.0 | NA | 100.0 | 0.0 | NA |
| | Tulathromycin | 0 | 199 | 0 | 0 | 100.0 | NA | 100.0 | NA | 100.0 | 0.0 | NA |
| | Total | 8 | 2121 | 1 | 5 | 99.7 | 61.5 | 100.0 | 88.9 | 99.8 | 0.0 | 38.5 |
| H-Ec | Amoxicillin | 63 | 22 | 0 | 1 | 98.8 | 98.4 | 100.0 | 100.0 | 95.7 | 0.0 | 1.6 |
| | Amoxicillin/clavulanic acid | 14 | 179 | 0 | 18 | 91.5 | 43.8 | 100.0 | 100.0 | 90.9 | 0.0 | 56.3 |
| | Ampicillin | 85 | 39 | 0 | 1 | 99.2 | 98.8 | 100.0 | 100.0 | 97.5 | 0.0 | 1.2 |
| | Cefotaxime | 7 | 117 | 1 | 0 | 99.2 | 100.0 | 99.2 | 87.5 | 100.0 | 0.8 | 0.0 |
| | Cefpodoxime | 0 | 86 | 0 | 0 | 100.0 | NA | 100.0 | NA | 100.0 | 0.0 | NA |
| | Ceftiofur | 0 | 124 | 0 | 1 | 99.2 | 0.0 | 100.0 | NA | 99.2 | 0.0 | 100.0 |
| | Chloramphenicol | 30 | 91 | 4 | 0 | 96.8 | 100.0 | 95.8 | 88.2 | 100.0 | 4.2 | 0.0 |
| | Ciprofloxacin | 14 | 105 | 6 | 0 | 95.2 | 100.0 | 94.6 | 70.0 | 100.0 | 5.4 | 0.0 |
| | Colistin | 2 | 180 | 29 | 0 | 86.3 | 100.0 | 86.1 | 6.5 | 100.0 | 13.9 | 0.0 |
| | Doxycycline | 46 | 24 | 15 | 1 | 81.4 | 97.9 | 61.5 | 75.4 | 96.0 | 38.5 | 2.1 |
| | Enrofloxacin | 0 | 78 | 0 | 8 | 90.7 | 0.0 | 100.0 | NA | 90.7 | 0.0 | 100.0 |
| | Florfenicol | 35 | 173 | 1 | 2 | 98.6 | 94.6 | 99.4 | 97.2 | 98.9 | 0.6 | 5.4 |
| | Gentamicin | 38 | 173 | 0 | 0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 0.0 | 0.0 |
| | Nalidixic acid | 4 | 115 | 6 | 0 | 95.2 | 100.0 | 95.0 | 40.0 | 100.0 | 5.0 | 0.0 |
| | Neomycin | 66 | 143 | 2 | 0 | 99.1 | 100.0 | 98.6 | 97.1 | 100.0 | 1.4 | 0.0 |
| | Spectinomycin | 117 | 61 | 24 | 9 | 84.4 | 92.9 | 71.8 | 83.0 | 87.1 | 28.2 | 7.1 |
| | Streptomycin | 161 | 27 | 20 | 3 | 89.1 | 98.2 | 57.4 | 89.0 | 90.0 | 42.6 | 1.8 |
| | Tetracycline | 135 | 71 | 4 | 1 | 97.6 | 99.3 | 94.7 | 97.1 | 98.6 | 5.3 | 0.7 |
| | Trimethoprim | 63 | 53 | 1 | 8 | 92.8 | 88.7 | 98.1 | 98.4 | 86.9 | 1.9 | 11.3 |
| | Trimethoprim/ sulfamethoxazol | 0 | 38 | 0 | 48 | 44.2 | 0.0 | 100.0 | NA | 44.2 | 0.0 | 100.0 |
| | Total | 880 | 1899 | 113 | 101 | 92.8 | 89.7 | 94.4 | 88.6 | 95.0 | 5.6 | 10.3 |

Data are based on epidemiological cut-offs (ECOFFs) and clinical breakpoints when ECOFFs are unavailable (https://www.vetssi.dk/)
Abbreviations: Ap, *Actinobacillus pleuropneumoniae*; H-Ec, haemolytic *Escherichia coli*; Ss, *Streptococcus suis;* P+, resistant phenotype; P-, susceptible phenotype; G+, resistant gentotype; G-, susceptible genotype; PPV, positive predictive value; NPV, negative predictive value; ME, major error; VME, very major error; NA, not applicable

**continued ... Table 9.4 Diagnostic performance of ResFinder 4.1 as an antimicrobial resistance prediction tool for pathogenic bacteria from pigs, Denmark, 2021-2022** DANMAP 2022

| Pathogen | Antimicrobial agent | P+/G+ | P-/G- | G+/P- | G-/P+ | Concordance | Sensitivity | Specificity | PPV | NPV | ME rate | VME rate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ss | Chloramphenicol | 0 | 183 | 2 | 1 | 98.4 | 0.0 | 98.9 | 0.0 | 99.5 | 1.1 | 100.0 |
| | Doxycycline | 12 | 33 | 0 | 10 | 81.8 | 54.5 | 100.0 | 100.0 | 76.7 | 0.0 | 45.5 |
| | Enrofloxacin | 0 | 55 | 0 | 0 | 100.0 | NA | 100.0 | NA | 100.0 | 0.0 | NA |
| | Erythromycin | 115 | 68 | 1 | 2 | 98.4 | 98.3 | 98.6 | 99.1 | 97.1 | 1.4 | 1.7 |
| | Florfenicol | 4 | 237 | 0 | 0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 0.0 | 0.0 |
| | Penicillin | 0 | 237 | 0 | 4 | 98.3 | 0.0 | 100.0 | NA | 98.3 | 0.0 | 100.0 |
| | Tetracycline | 53 | 103 | 0 | 20 | 88.6 | 72.6 | 100.0 | 100.0 | 83.7 | 0.0 | 27.4 |
| | Trimethoprim/ sulfamethoxazol | 0 | 198 | 0 | 43 | 82.2 | 0.0 | 100.0 | NA | 82.2 | 0.0 | 100.0 |
| | Total | 184 | 1114 | 3 | 80 | 94.0 | 69.7 | 99.7 | 98.4 | 93.3 | 0.3 | 30.3 |

Data are based on epidemiological cut-offs (ECOFFs) and clinical breakpoints when ECOFFs are unavailable (https://www.vetssi.dk/)
Abbreviations: Ap, *Actinobacillus pleuropneumoniae*; H-Ec, haemolytic *Escherichia coli*; Ss, *Streptococcus suis;* P+, resistant phenotype; P-, susceptible phenotype; G+, resistant gentotype; G-, susceptible genotype; PPV, positive predictive value; NPV, negative predictive value; ME, major error; VME, very major error; NA, not applicable

## 9.5 Conclusions and perspectives

AST showed that most pathogenic bacteria from pigs displayed similar frequencies of phenotypic resistance as in previous years.

The high frequency of resistance in haemolytic *E. coli* to first-choice drugs for treatment of *E. coli*-associated post-weaning diarrhoea is worrisome and should be monitored closely in the coming years. Of note, our interpretation was based on ECOFFs as animal-specific clinical breakpoints for these drugs are currently lacking. ECOFFs are based on microbiological studies and do not necessarily indicate whether a drug will be clinically active. Future studies should therefore seek to establish animal-specific clinical breakpoints to antimicrobial agents of veterinary importance by considering what happens to the drug within a specific animal and body site (pharmacokinetics).

WGS demonstrated that resistance towards antimicrobial agents considered critically important for human medicine remained at a low level.

WGS seems to be a promising tool for prediction and surveillance of AMR in pathogenic bacteria from pigs. However, it was not always possible to compare AST and WGS results due to the lack of ECOFFs and clinical breakpoints for many antimicrobial agents of veterinary importance, and due to limited knowledge on genes and mutations conferring resistance to these drugs. In addition, the ResFinder 4.1 genotype-to-phenotype translation scheme for point mutations in *K. pneumoniae* is under development and the phenotypes are currently based on antimicrobial classes rather than agents, which might explain some of its poor performance in this species. Closing these gaps could substantially improve the usefulness of WGS for AMR prediction and surveillance in pathogenic bacteria from animals. WGS is also a useful tool for monitoring resistance mechanisms in pathogenic bacteria, for which AST is unavailable, and for tracing the spread of specific resistance genes and pathogenic bacteria within and between animal and human populations.

It has been agreed not to mention additional material in this year's report.

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## Textbox 9.1

# Antimicrobial resistance in dogs and cats: focus on extended-spectrum cephalosporinase-producing *Escherichia coli* and their resemblance to human clinical isolates

**Background**

In Denmark, reports on antimicrobial resistance in canine and feline pathogens have been published sporadically over the years, last time in DANMAP 2019. Traditionally, susceptibility results have been reported for *Escherichia coli* and *Staphylococcus pseudintermedius*, which are the most frequently isolated bacterial pathogens of companion animals and the primary causes of urinary tract and skin infections, respectively. These species can have important resistant and multidrug-resistant phenotypes. Of particular concern are extended-spectrum cephalosporinase- (ESC) producing *E. coli* and methicillin-resistant *S. pseudintermedius* (MRSP), which have emerged worldwide in the last two decades and constitute a threat to animal health, as they can be resistant to all veterinary licensed antibiotics [1]. *E. coli* and to a lesser extent MRSP are also pathogens of public health relevance due to the risk of zoonotic transmission.

**Materials and Methods**

Antimicrobial susceptibility data were retrieved for 726 *S. pseudintermedius* and 559 *E. coli* isolates obtained in 2020-2022 from various infections in dogs and cats (Table 1). Diagnostic specimens had been shipped from primary care and referral veterinary hospitals from across Denmark to the diagnostic laboratory Sund Vet Diagnostik at the University of Copenhagen. Susceptibility testing was done using broth microdilution with commercial Sensititre plates (ThermoFisher Scientific). Interpretation of MIC data was according to clinical breakpoints published by the Clinical and Laboratory Standards Institute [2]. Sixteen cefpodoxime-resistant *E. coli* isolates stored in the period 2012-2022 were subjected to Illumina MiSeq sequencing, followed by genome assembly using SPAdes v.3.13.1 [3]. Multi-locus sequence typing and screening for genes encoding ESBL or plasmid-borne AmpC genes was performed using mlst v2.19.0 (https://github.com/tseemann/mlst), and Abricate v1.0.1. (https://github.com/tseemann/abricate), respectively. Sequences were then imported into the software SeqSphere+ (Ridom) for construction of core-genome (CG) MLST phylogeny based on analysis of 2,513 genes. Here, cgMLST profiles were compared to corresponding profiles of all 1,243 Danish human ESBL/AmpC-producing *E. coli* isolates obtained from blood infections in 2014-2023 and representing the sequence types (STs) found in dogs and cats.

**Table 1 Origin of *E. coli* and *S. pseudintermedius* isolates obtained from clinical specimens in Sund Vet Diagnostik, 2020-2022**

DANMAP 2022

| | *Staphylococcus pseudintermedius* | | *Escherichia coli* | |
|---|---|---|---|---|
| | Dogs | Cats | Dogs | Cats |
| Skin, wounds and ears | 594 | 13 | 114 | 16 |
| Urinary tract | 48 | 1 | 288 | 73 |
| Other | 68 | 2 | 56 | 12 |
| Total | 710 | 16 | 458 | 101 |

**Results and Discussion**

Except for minor fluctuations, levels of antimicrobial resistance were very similar to those encountered in the latest surveillance periods, namely 2016-17 and 2018-19 (Table 2). One example to highlight for *S. pseudintermedius* is oxacillin (6%), which is used as diagnostic indicator for MRSP. In *E. coli*, no isolates displayed carbapenem resistance, whereas 4% and 3% of isolates were resistant to fluoroquinolone and 3rd generation cephalosporin (3GC), respectively. The latter drug class (3GC) is used as diagnostic indicator for ESC production (i.e. ESBLs and AmpCs), hence the level of these resistant bacteria remains stable or may even be decreasing slightly. Sequencing of 3GC-resistant *E. coli* revealed that CMY-2 and CTX-M-15 are the most common ESCs, being present in 7 (44%) and 4 (25%) isolates, respectively. This is in line with previous studies reporting these to be among the predominant ESCs in companion animals [4;5]. Five of seven CMY-2-producing isolates belonged to ST372,

**continued ... Textbox 9.1**

which has recently been described as a major dog-adapted *E. coli* lineage [5]. The second most common sequence type was ST131 (n=3), which in humans predominates as a multi-resistant and hyper-virulent CTX-M-15-producing lineage, typically of the O25:4 *fimH*30 variant. Only one of the three canine ST131 isolates was positive for CTX-M-15. Although this strain was multi-resistant and carried 36 predicted virulence genes, the strain was classified as O16:J5 *fimH*41, hence different from the worldwide dominating ST131 lineage, and different from human clinical isolates in Denmark.

Using an arbitrary cut-off of 30 alleles, we found by cgMLST i) 2 allele differences between the canine ST155/DHA-1 isolate and a human isolate, ii) 21-25 allele differences between the canine ST162/CTX-M-15 isolate and three human isolates, and iii) 8 allele differences between the canine ST131/CTX-M-27 isolate and one human isolate. Remaining dog and cat isolates had between 32 and several hundred allele differences to human isolates. It is unlikely that any of the ESBL/AmpC-producing isolates originate from pets and humans living together. Nevertheless, these findings support previous research indicating that pets may be reservoirs of human-infectious *E. coli* lineages, and that zoonotic transmission within households is possible [6;7]. In extension to that, a recent Danish study indicated that approximately one out of ten dog owners with community-associated UTI share the infectious *E. coli* strain with their dog [6].

**Table 2 Percentages of antimicrobial-resistant clinical *E. coli* and *S. pseudintermedius* isolates from dogs and cats in Denmark**

DANMAP 2022

| | *Escherichia coli* | | | *Staphylococcus pseudintermedius* | | |
|---|---|---|---|---|---|---|
| Antimicrobial agent | 2016-2017 (N=394) | 2018-2019 (N=441) | 2020-2022 (n=559) | 2016-2017 (N=486) | 2018-2019 (N=602) | 2020-2022 (n=726) |
| | % | % | % | % | % | % |
| Amikacin | 2 | 2 | 1 | 1 | 1 | 1 |
| Ampicillin [1] | 14 | 25 | 22 | 59 | 70 | 65 |
| Amoxicillin/clavulanic acid [1] | 4 | 5 | 6 | 8 | 7 | 6 |
| Cefazolin | - | - | - | 8 | 7 | 6 |
| Cefpodoxime | 4 | 5 | 3 | - | - | - |
| Chloramphenicol | 4 | 4 | 2 | 16 | 21 | 20 |
| Clindamycin | - | - | - | 25 | 27 | 26 |
| Doxycycline | 7 | 8 | 4 | 33 | 29 | 28 |
| Enrofloxacin | 3 | 4 | 4 | 3 | 2 | 4 |
| Erythromycin | - | - | - | 26 | 28 | 26 |
| Gentamicin | 4 | 4 | 3 | 3 | 2 | 5 |
| Imipenem | 0 | 0 | 0 | - | - | - |
| Marbofloxacin | 3 | 3 | 4 | 3 | 3 | 4 |
| Oxacillin | - | - | - | 8 | 6 | 6 |
| Sulfamethoxazole/trimethoprim | 7 | 9 | 8 | 5 | 6 | 8 |

1) Susceptibility data for ampicillin and amoxicillin/clavulanic acid in *E. coli* have been determined only for isolates from urinary tract infections, as isolates from other infections are unequivocally classified as resistant to these drugs according to CLSI breakpoints

**Table 3. Multilocus sequence types and extended-spectrum cephalosporinases detected in the 16 stored *E. coli* isolates resistant to cefpodoxime**
DANMAP 2022

| Isolate | Extended-spectrum cephalosporinase | Sequence type | Origin | Year |
|---|---|---|---|---|
| 1 | CMY-2 | ST372 | Dog | 2012 |
| 2 | CTX-M-14 | ST448 | Dog | 2015 |
| 3 | CTX-M-27 | ST131 | Dog | 2015 |
| 4 | CMY-2 | ST963 | Dog | 2016 |
| 5 | CTX-M-15 | ST648 | Cat | 2017 |
| 6 | CMY-2 | ST372 | Dog | 2018 |
| 7 | CMY-2 | ST372 | Dog | 2019 |
| 8 | CTX-M-15 | ST131 | Dog | 2019 |
| 9 | CMY-2 | ST372 | Dog | 2019 |
| 10 | CMY-2 | ST14967 | Dog | 2019 |
| 11 | CMY-2 | ST372 | Dog | 2021 |
| 12 | DHA-1 | ST155 | Dog | 2021 |
| 13 | CTX-M-15 | ST998 | Dog | 2022 |
| 14 | CTX-M-1 | ST88 | Dog | 2022 |
| 15 | CTX-M-3 | ST131 | Dog | 2022 |
| 16 | CTX-M-15 | ST162 | Dog | 2022 |

## Conclusion

Antimicrobial resistance in clinical *E. coli* and *S. pseudintermedius* from dogs and cats in Denmark has been stable over the last six years. The close genetic similarities between canine and human clinical ESC-producing *E. coli* isolates indicates a potential risk of transmission between the two hosts. Further research is needed to understand if the close genetic similarities detected are limited to ESC-producing strains like ST131, and if this and other human pathogenic lineages also circulate in the canine population as non-ESC producers.

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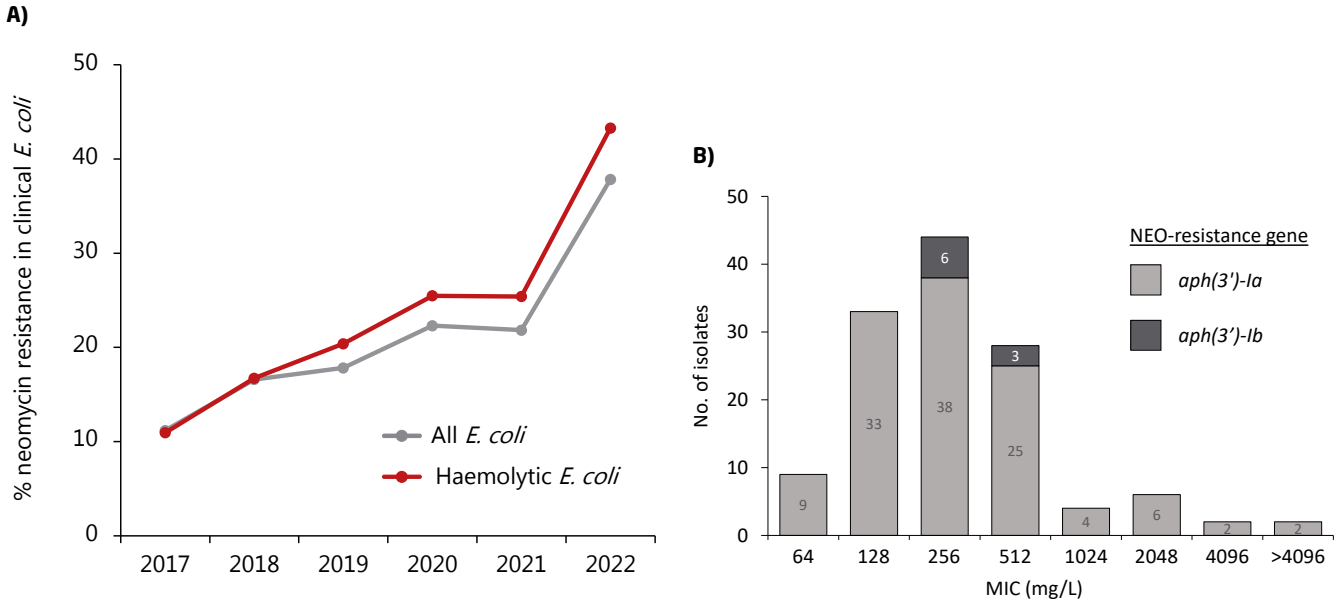
## Textbox 9.2

# Insights into the genetic basis of neomycin resistance in clinical *Escherichia coli* isolated from pigs

### Background

Neomycin is commonly used as a first choice antibiotic for treating porcine enteritis caused by enterotoxigenic *Escherichia coli* (ETEC). After the ban on zinc oxide, a rise in neomycin resistance has been observed in Denmark (Figure 1A), likely due to increased neomycin use [1]. In this study, we elucidated the mechanisms of neomycin resistance by characterizing a collection of 128 neomycin-resistant clinical *E. coli* isolated from Danish pig farms between 2015 and 2020 [2].

**Figure 1 Prevalence of neomycin-resistance among porcine clinical *E. coli* isolates after the reintroduction of neomycin in 2017 (A) and MIC distribution in the 128 neomycin-resistant strains analyzed in this study (B)** DANMAP 2022




Source: Danish Agriculture and Food Council, Veterinary Laboratory, Kjellerup

### Methods

All isolates were analysed by Illumina sequencing and antimicrobial susceptibility testing. Conjugation experiments were performed on 32 strains selected based on phylogenetic analysis to assess plasmid transferability. For further understanding of the structures and associated mobile genetic elements in the plasmids encoding neomycin resistance, eight selected representative strains were subjected to long-read sequencing using Nanopore.

### Results

We identified 35 different *E. coli* lineages with sequence types ST100 (38.3%) and ST10 (22.7%) accounting for approximately 61% of the isolates. While ST100 was strongly associated with ETEC displaying fimbria type F4, ST10 occurred in both ETEC with fimbria type F18 and non-ETEC strains (Figure 2). Most isolates (95.3%) were resistant to three or more antimicrobial classes in addition to neomycin. The MICs of neomycin were extremely variable (64 to ≥4096 mg/L) with most isolates (82%) displaying MICs of 128-512 mg/L (Figure 1B). Neomycin resistance was transferable under laboratory conditions from 25 out of the 32 selected strains. The genes encoding neomycin resistance were *aph(3')-Ia* (93%) and *aph(3')-Ib* (7%). While the former gene was associated with two types of transposons, Tn903 or Tn4352, which were distributed on a variety of conjugative plasmid backbones (mainly IncI1α but also IncHI1, IncHI2, IncN and ColRNAI), the second gene was not flanked by any transposable element and was consistently found on a small (19 kb) non-conjugative but mobilizable plasmid that was traced back to distantly related Gram-negative bacteria like *Achromobacter* and *Pseudomonas putida*.

**Figure 2 Phylogenetic tree of the 128 sequenced neomycin-resistant *E. coli* isolates using core-genome alignment. The tree displays neomycin resistance gene, sequence type (ST), ETEC status and fimbrial type for each strain (modified from reference [2])**

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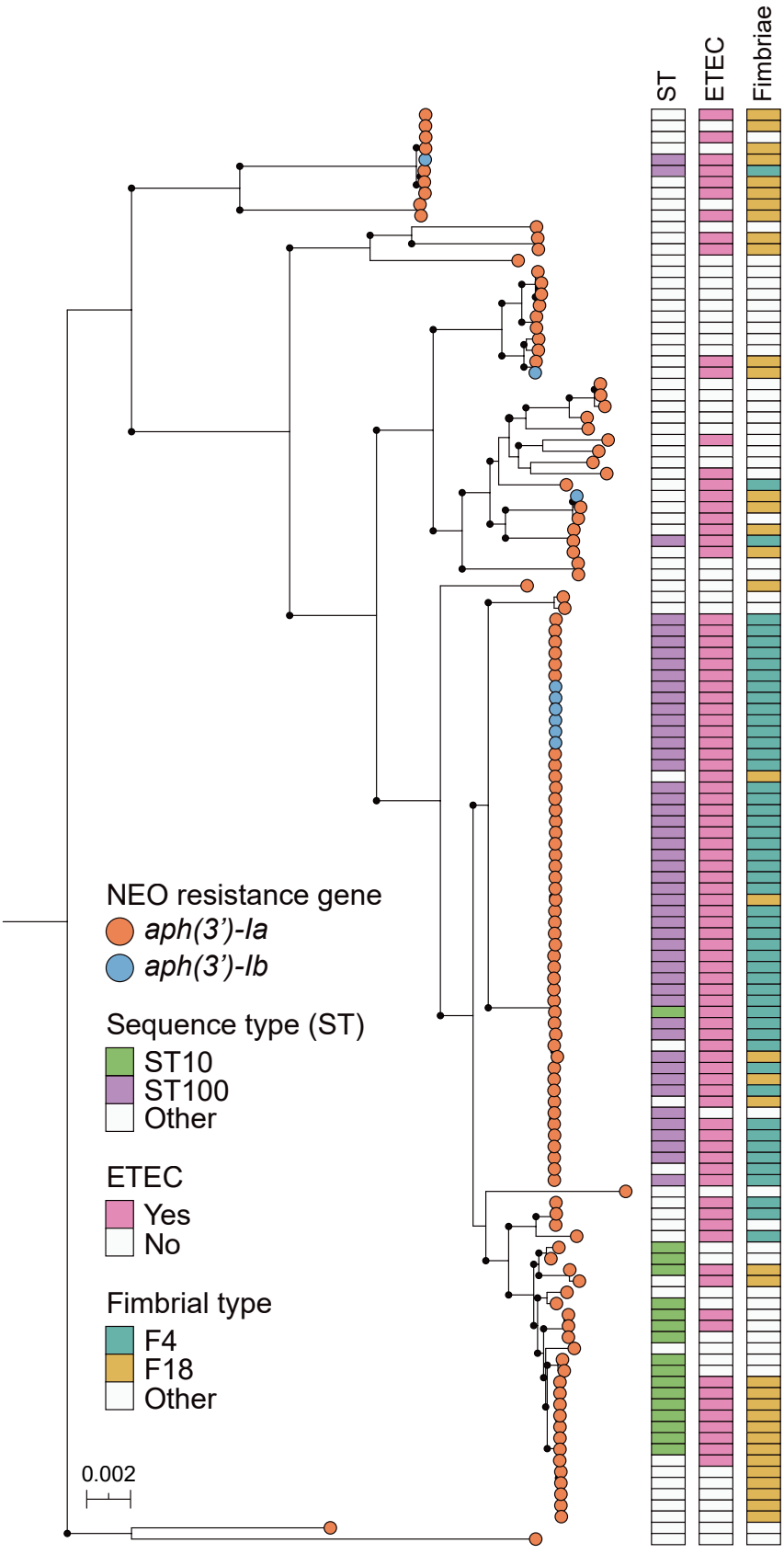

continued ... Textbox 9.2

**Discussion**

The results show that the spread of neomycin resistance recently observed in clinical *E. coli* from Danish pig farms is driven by two resistance determinants that are located on different plasmid scaffolds capable of spreading across many different *E. coli* lineages. The two most common lineages, ST100 and ST10, have previously been reported as prevalent among clinical porcine ETEC strains in various regions of the world, including Denmark [3]. The latter lineage has zoonotic potential, since it is one of the five most common epidemic lineages responsible for human extraintestinal infections globally [4].

Neomycin-resistant strains displayed high rates of resistance to alternative antibiotics that can be used to manage porcine ETEC enteritis, such as spectinomycin (89.8%), sulfamethoxazole (85.9%), and tetracycline (78.9%). This result highlights the lack of effective alternatives to neomycin for treatment of this common disease in pig production. The situation will unlikely improve in the future, since our study shows that *aph(3')-Ia* is usually located on plasmids carrying genes conferring resistance to other antimicrobials such as tetracyclines, providing evidence that neomycin resistance may be co-selected by the use of other antimicrobials and vice versa. The lack of effective alternatives to neomycin underscores the importance of implementing strategies to preserve the efficacy of neomycin and explore novel approaches to managing this common infection in pig production, including alternatives to antimicrobials (see Chapter 4, Textbox 4.3).

This study provides valuable insights into the genetic basis of neomycin resistance in porcine clinical *E. coli* strains. In the absence of a validated clinical breakpoint, it is still unclear if all strains tested *in vitro* as neomycin-resistant are *in vivo* resistant, especially due to the low oral bioavailability of this aminoglycoside and the high concentrations achieved in the intestinal tract following oral administration [5]. The high variability of MICs observed in this study highlights the importance of assessing the clinical efficacy of neomycin in the field while monitoring the evolution of the neomycin resistance phenotype in the years to come.

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## Textbox 9.3

# Assessing the burden of Antimicrobial Resistance and Usage in the Global Burden of Animal Disease programme: the start of the Danish case study

**Background**

The Global Burden of Animal Diseases programme (GBADs) [1] is an international collaboration of partners that aims to assess the burden of animal disease from an economic perspective in terms of net loss of production, expenditure, and impacts on the economy and trade within the context of food systems. It measures the burden of disease in livestock in terms of Animal Health Loss Envelope (AHLE), an approach to calculate the absolute cost of disease against a zero-cost ideal [2].

This overall loss envelope can then be disaggregated into specific causes, including infectious diseases and within those, antimicrobial resistance (AMR). A better understanding of the socio-economic impact of AMR and of antimicrobial usage (AMU) in livestock is key to efficiently tackling the threat of AMR. Burden data provides baseline information and underpins cost-effectiveness assessments for changes in practices. Yet gaps remain in our understanding, including how AMR leads to livestock production and animal health losses, veterinary expenditure, and externalities in public health and the environment. The GBADs' work on AMU and AMR aims to address these gaps, by providing a methodology nested within the AHLE framework, identifying data requirements, and generating burden estimates in selected case studies.

In the Danish case study, the component of the AHLE attributable to AMU and AMR in the pig sector will be estimated. As the first GBADs case study on AMU/AMR, the results will be particularly relevant for further applications of the methodology. Lessons learned will be used to refine the analytical framework developed and to better understand data challenges. This textbox expands on the analytical approach that is being taken for this assessment and describes the next stages of the work.

**Analytical approach**

AMR and AMU can contribute to the AHLE component attributable to infectious diseases through different pathways. On one hand, the usage of antimicrobials in farmed animals constitutes a component of the health expenditure accrued when mitigating or preventing infectious diseases' impact. On the other hand, AMR's potential negative effects on the severity and/or duration of illness in animals will be a contributor to mortality and productivity losses associated with infectious diseases. If treated, those resistant infections will contribute further to the AHLE as additional healthcare expenditure due to treatment failures, including repeated treatments and treatment with potentially costlier therapeutic alternatives. Figure 1 summarizes these pathways for losses.

**Methods and next steps in the Danish case study**

The Danish case study will follow the analytical framework described above and will focus specifically on the pig sector.

The first stage of the assessment is focusing on the assessment of the AMU burden. Data on antimicrobial consumption and sales for 2021 in pigs has been sourced from the VetStat database. Expenditure is currently being estimated by combining the VetStat data on consumption and sales and pricing data published by Medicin Til Dyr [3]. The pricing data has been extracted using Web Scraping scripts developed to automate the process. Web Scraping scripts were developed in Python using the Selenium package [4] and are available in the GBADs GitHub [5].

The work will move to assess the AMR burden, in terms of contribution to mortality and productivity losses, and extra health care expenditure within the AHLE, with data inputs from DANMAP. Currently available AHLE estimates for swine production in Denmark [6] will also be refined in the next stages.

continued ... Textbox 9.3

**Figure 1 Analytical approach used to estimate the burden of AMR and AMU in the Global Burden of Animal Disease programme**

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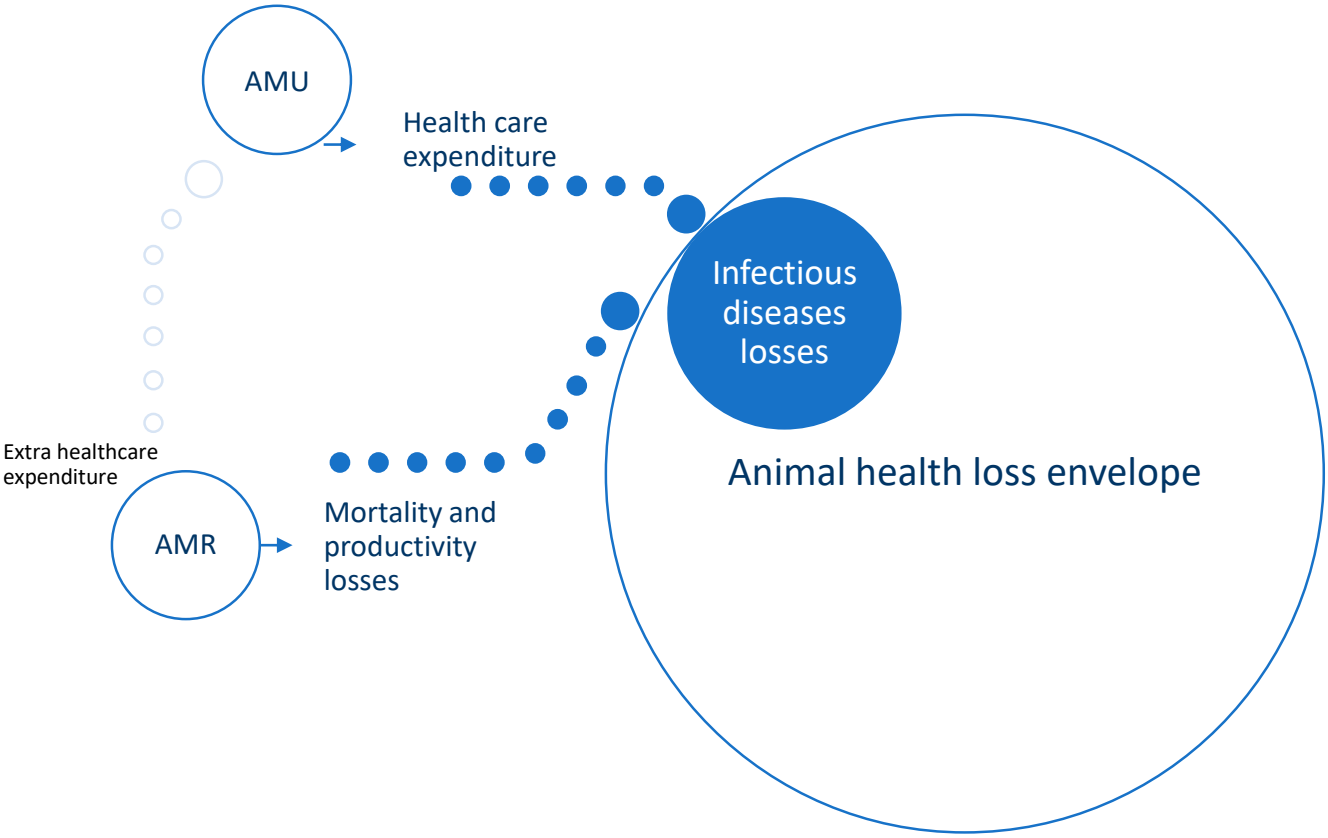


The animal health loss envelope includes mortality and morbidity-associated losses and healthcare expenditure attributable to infectious diseases, non-infectious diseases and external hazards. AMU: antimicrobial usage; AMR: antimicrobial resistance

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