



6

RESISTANCE IN
ZONOTIC BACTERIA



6. Resistance in zoonotic bacteria



Highlights: In 2022, compared to 2021, the number of *Campylobacter jejuni* (285) and *Salmonella* Typhimurium (190) isolates from humans tested for antimicrobial susceptibility decreased, although the number of outbreaks increased, from three to ten for *C. jejuni* and from three to six for *S. Typhimurium*. The number of tested travel-associated isolates also increased in 2022 for both bacteria, from 29 to 52 for *C. jejuni* and from 13 to 30 for *S. Typhimurium*. The number of *Salmonella* Typhimurium isolates recovered from Danish pork increased in 2022, from 39 to 48.

As in previous years, the occurrence of resistance in ***C. jejuni*** isolated from humans was higher than in isolates recovered from broilers and cattle. Among human infections, resistance was higher in travel-associated compared to domestically-acquired cases.

Compared to 2021, the percentage of fully-sensitive *C. jejuni* decreased in isolates from broilers (from 73% to 59%) and increased in isolates from cattle (from 68% to 76%).

In 2022, resistance to erythromycin, chloramphenicol and gentamicin was not detected in any of the monitored *Campylobacter* isolates. Like in 2021, ertapenem resistance was observed in 1% and 12% of isolates from domestic- and travel-related cases, respectively (Table 6.1), and in 2% of isolates from broilers.

Fluoroquinolone (ciprofloxacin) resistance remained common in *C. jejuni* isolates obtained from human cases (58%), broilers (38%) and cattle (22%). Resistance towards tetracycline was common in *C. jejuni* from humans and broilers with 29% and 24% of resistant isolates, respectively, whereas only 5% of isolates from cattle were tetracycline-resistant. Contrary to the decrease observed in the latest years, ciprofloxacin resistance and combined resistance to ciprofloxacin and tetracycline increased in *C. jejuni* isolates from broilers.

C. coli from broilers were commonly resistant to ciprofloxacin (39%) and tetracycline (45%). The percentage of fully-sensitive isolates (38%) was lower than for *C. jejuni*, and 23% *C. coli* exhibited resistance to ertapenem.

The level of azithromycin resistance in ***Salmonella* Typhimurium, including the monophasic variants**, was less than 1% in human isolates, and 4% in isolates from Danish pork. Ciprofloxacin resistance was observed in 4% of the isolates from domestically-acquired infections and in 20% of travel-associated cases. Historically, ciprofloxacin resistance has predominantly been observed in isolates from travel-associated cases. Fluoroquinolone resistance has not been recorded in *S. Typhimurium* from Danish pork since 2007.

Resistance to the critically important 3rd generation cephalosporins and carbapenems is rare in *S. Typhimurium*. In 2022, third-generation cephalosporin resistance was observed in 1% of the human isolates and no carbapenem resistance was observed. In line with the previous years, resistance to 3rd generation cephalosporins and carbapenems was not observed in *S. Typhimurium* isolates from Danish pork.

6.1 Introduction

6.1.1 Resistance in zoonotic bacteria

Zoonoses are infectious diseases that can be transmitted between animals and humans, either through direct contact or indirectly by ingestion of contaminated food or water, or contact with contaminated environment. *Campylobacter* and *Salmonella* are a common cause of zoonotic gastrointestinal infections and are therefore considered a key component of zoonotic monitoring programmes. A description of the trends and sources of these zoonoses in Denmark and of national surveillance and control programmes can be found in the Annual Report on Zoonoses in Denmark 2022 [www.food.dtu.dk]. Surveillance of antimicrobial resistance (AMR) in *Campylobacter* and *Salmonella* from food-producing animals, meat and humans has been part of the DANMAP programme since 1995. Phenotypic antimicrobial resistance is monitored in isolates from human clinical cases, broilers, cattle, pigs and corresponding meat. Additionally, whole genome sequencing (WGS) is an integrated part of the surveillance of human *Campylobacter* and *Salmonella* infections in Denmark, and is performed on a selection of *Campylobacter* and *Salmonella* isolates recovered from food-producing animals. Hence, monitoring of genotypic antimicrobial resistance in these bacteria may soon become an integral part of DANMAP (see Textbox 6.1).

In Denmark, antimicrobials are generally not recommended for treatment of diarrhoea in human patients unless there is prolonged duration of disease or the patient is severely ill. If treatment is required, macrolides (azithromycin) are recommended for treatment of *Campylobacter* infections. For *Salmonella* infections, no specific recommendations regarding antibiotic treatment exist for the primary sector. For infections treated in hospitals, intravenous ceftriaxone is recommended for septic patients and per oral azithromycin for less severe cases. For prolonged or recurrent infections with *Salmonella*, combination therapy can be used, with ciprofloxacin or sulfamethoxazole and trimethoprim added. The Register of Medicinal Product Statistics at the Danish Health Data Authority does receive information on the indication for prescribing an antibiotic, but not against which pathogen it was prescribed.

Macrolides are often used to treat infections in food-producing animals in Denmark, especially in pigs. Fluoroquinolones are not used in any production animals, whereas there is a limited use of 2nd generation cephalosporins and no use of 3rd and 4th generation cephalosporins in cattle. The use of antimicrobials in the Danish poultry sector is low and limited to only a few antimicrobial classes, primarily tetracyclines (see Chapter 4, Table 4.1).

In humans, monitoring of antimicrobial resistance is performed on clinical isolates of *Salmonella*. For *Campylobacter jejuni* a geographically stratified selection of isolates is subjected to susceptibility testing. The testing is performed in accordance with the ECDC recommendations (see Chapter 10, Section 10.9). Travel histories of the patients are collected, when possible.

Campylobacter isolates were obtained from healthy animals at slaughter (caecal samples from broilers and cattle), while *Salmonella* isolates were obtained from pig carcasses at slaughter. *C. jejuni* is reported for broilers and cattle, and *C. coli* is reported for broilers (see Chapter 10, Table 10.1 for further details). Since 2021, the antimicrobial susceptibility testing of *Campylobacter* and *Salmonella* from animals and meat has been done in accordance with the Commission Implementing Decision 2020/1729/EU of 17 November 2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (see Chapter 10 for further details).

6.2 *Campylobacter*

A total of 285 human *C. jejuni* isolates were susceptibility tested. The isolates represented 233 domestically-acquired infections and 52 travel-associated infections and included 33 outbreak related isolates from ten different outbreaks. A large number of sequence types (ST) were identified among the susceptibility tested strains with ST21 (41), ST50 (23) and ST52 (21) as the predominant STs.

All *C. jejuni* isolates recovered from broilers (170), most *C. jejuni* recovered from cattle (102), and all *C. coli* isolates recovered from broilers (56) were susceptibility tested.

6.2.1 Resistance in *Campylobacter jejuni*

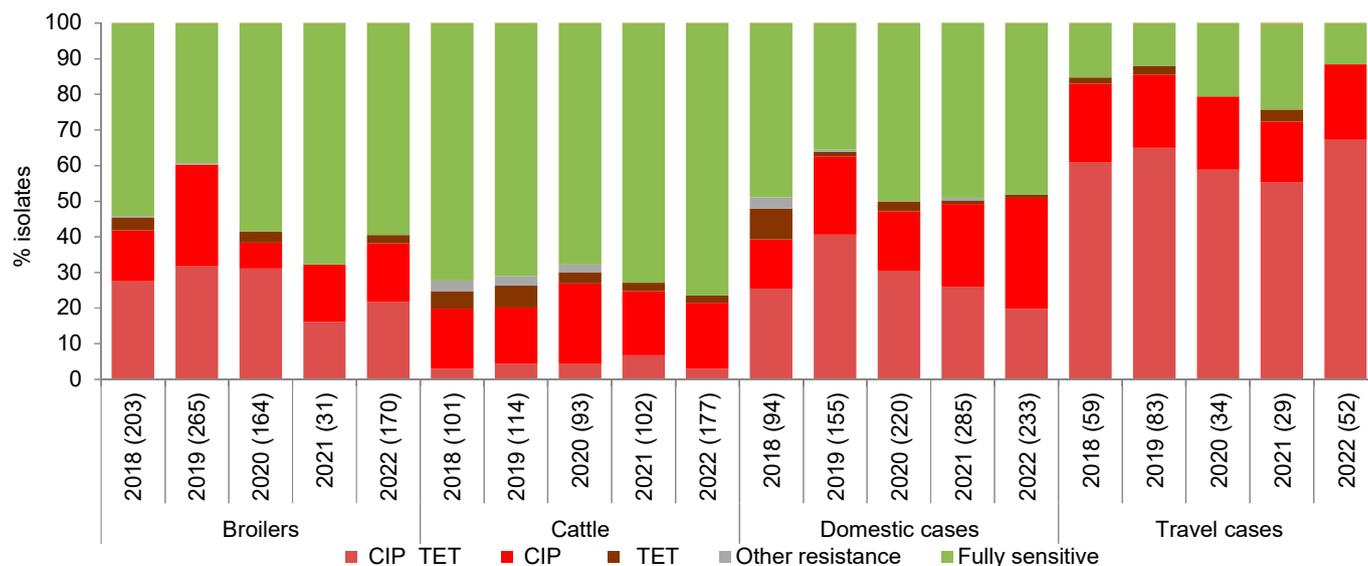
The levels of resistance in *C. jejuni* isolates from humans, and from Danish broilers and cattle at slaughter are presented in Table 6.1. Resistance was common for ciprofloxacin and tetracycline in isolates from humans, broilers, and cattle, whereas no resistance to chloramphenicol, erythromycin and gentamicin was observed in 2022. Ertapenem resistance has been monitored since 2021 and the levels of ertapenem resistance in human isolates were in line with the findings of the previous year. A total of 3% of the isolates, with 1% and 12% of isolates from domestic- and travel-related cases, respectively, showed ertapenem resistance. Unlike 2021, in 2022 ertapenem resistance was also found in 2% of *C. jejuni* isolates from broilers (Table 6.1).

In 2022, 48% from domestically-acquired human cases, 59% of *C. jejuni* from broilers, and 76% from cattle were sensitive to all antimicrobials tested. The percentage of fully-sensitive *C. jejuni* isolates from domestically-acquired human infections was in line with the previous years and likewise the resistance levels were comparatively higher in infections from travel-related cases.

Unlike the previous year, the percentage of fully-sensitive *C. jejuni* isolates from broilers decreased in 2022, while the percentage of full-sensitivity among isolates from cattle has remained relatively constant in the past five years (Figure 6.1).

Figure 6.1 Distribution (%) of AMR profiles in *Campylobacter jejuni* from broilers, cattle and human cases, Denmark

DANMAP 2022



The number of isolates included each year is shown in parentheses. A human isolate is categorised as domestically-acquired if the patient did not travel outside Denmark one week prior to the onset of disease. CIP: all isolates with ciprofloxacin resistance but not tetracycline resistance, TET: all isolates with tetracycline resistance but not ciprofloxacin resistance, CIP TET: all isolates with both ciprofloxacin and tetracycline resistance, Other resistance: all isolates with neither ciprofloxacin- nor tetracycline resistance, Fully-sensitive: all isolates susceptible to all antimicrobial agents included in the test panel. CIP TET, CIP and TET isolates may be also resistant to other antimicrobials in the test panel (see Table 6.1)

Table 6.1 Resistance (%) in *Campylobacter jejuni* isolates from broilers, cattle and human cases, Denmark

DANMAP 2022

| Antimicrobial agent | Broilers | | Cattle | | Human | | Total % |
|---------------------|----------|----------|-------------------------|--------------------------|---------|--|---------|
| | Danish % | Danish % | Domestically acquired % | Travel abroad reported % | Total % | | |
| Chloramphenicol | 0 | 0 | 0 | 0 | 0 | | |
| Ciprofloxacin | 38 | 22 | 51 | 88 | 58 | | |
| Ertapenem | 2 | 0 | 1 | 12 | 3 | | |
| Erythromycin | 0 | 0 | 0 | 0 | 0 | | |
| Gentamicin | 0 | 0 | 0 | 0 | 0 | | |
| Tetracycline | 24 | 5 | 21 | 67 | 29 | | |
| Fully sensitive (%) | 59 | 76 | 48 | 12 | 41 | | |
| Number of isolates | 170 | 102 | 233 | 52 | 285 | | |

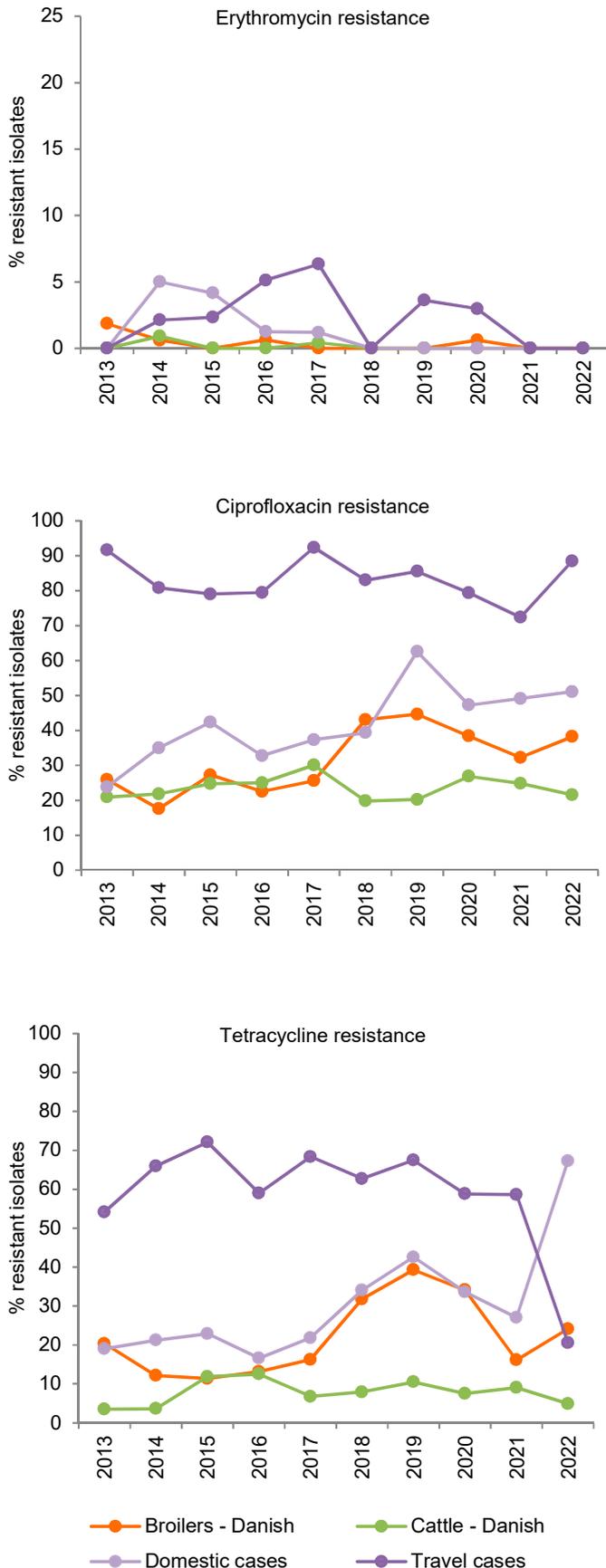
An isolate is categorised as domestically-acquired if the patient did not travel outside Denmark one week prior to the onset of disease
Fully-sensitive: all isolates susceptible to all antimicrobial agents included in the test panel

In 2022, the percentage of *C. jejuni* isolates from broilers with resistance to ciprofloxacin but not tetracycline was the same as in the previous year (16%), while the percentage of isolates with combined resistance to ciprofloxacin and tetracycline increased (from 16% to 22%). In cattle, *C. jejuni* showed a decrease in the combined resistance to ciprofloxacin and tetracycline (from 7% to 3%), compared to 2021. Resistance to tetracycline but not ciprofloxacin remained rare in isolates from humans and animals in 2022, as did resistance to antimicrobials other than ciprofloxacin and/or tetracycline (Figure 6.1).

As in previous years, the occurrence of resistance to ciprofloxacin and tetracycline in 2022 was higher in travel-associated isolates (88% and 67%, respectively) than in isolates from domestically-acquired infections (51% and 21%, respectively), and it was higher in human clinical isolates than in isolates from broilers and cattle (Figure 6.2).

Over the last decade, and until 2019, ciprofloxacin resistance has overall increased in *C. jejuni* from Danish broilers. This trend shifted to a decrease in 2020 and 2021 (note that the number of isolates was comparably lower in 2021 (N=31), which contributed to a higher uncertainty in the estimated occurrence of resistance in that year). However, in 2022 the occurrence of ciprofloxacin resistance increased again to the level of 2020 (38%) (Figure 6.2). As previously observed, the shift in the trend of resistance to ciprofloxacin coincided with the shifts in resistance to tetracycline (Figure 6.2) and combined resistance to both antibiotics (Figure 6.1). Fluoroquinolones are not used in food-producing animals in Denmark, suggesting that the development and spread of ciprofloxacin resistance in *C. jejuni* in broilers is driven by other mechanisms than direct antimicrobial use.

Figure 6.2 Erythromycin, ciprofloxacin and tetracycline resistance (%) among *Campylobacter jejuni* from broilers, cattle and human cases, Denmark DANMAP 2022



An isolate is categorised as domestically-acquired if the patient did not travel outside Denmark one week prior to the onset of disease

Macrolide resistance in *Campylobacter* is monitored using erythromycin. Erythromycin resistance was not observed in any *C. jejuni* isolates in 2022. Similarly, gentamicin resistance has been low or absent among *C. jejuni* from human isolates and has not been observed among *C. jejuni* from broilers and cattle in the last 10 years of monitoring. As in 2021, resistance to chloramphenicol was not observed among any of the tested *C. jejuni* isolates from humans or animals (Table 6.1).

Like in 2021, ertapenem resistance was observed in 1% and 12% of isolates from domestic- and travel-related cases, respectively, and in 2022 also in 2% of isolates from broilers (Table 6.1).

6.2.2 Resistance in *Campylobacter coli*

Erythromycin-, gentamicin- and chloramphenicol- resistance were not observed in *C. coli* isolates from broilers in 2022, similarly to what was observed in *C. jejuni* isolates. Resistance to ciprofloxacin in *C. coli* was also at a similar level as the one observed in *C. jejuni* (39%), while the occurrence of tetracycline resistance was comparatively higher in *C. coli* (45%). Resistance to ertapenem was detected in 23% of the *C. coli* isolates, also comparatively higher than the occurrence in *C. jejuni*.

6.2.3 Perspectives

Data from the two years of monitoring of ertapenem resistance in *C. jejuni* and *C. coli* from food-producing animals in many EU Member States, including Denmark, have shown possible different resistance wild-type distributions between the two *Campylobacter* species, as well as between different animal populations. This observation has been discussed between the European Food Safety Authority (EFSA), the European Centre for Disease Control (ECDC), the European Reference Laboratory for Antimicrobial Resistance (EURL-AR), the European Committee on Antimicrobial Susceptibility Testing (EUCAST), and many AMR academic experts.

It has been concluded that several points regarding ertapenem resistance in *Campylobacter* need to be clarified. A collaborative project has thus been initiated between EURL-AR, EFSA, ECDC and EUCAST in order to assess if the present epidemiological cut-off value (ECOFF) for interpretation is set correctly, if ertapenem is the best carbapenem to be included in the antimicrobial test panel, the effect of using different recommended test media, if there are differences in the wild-type resistance distribution between *Campylobacter* species and animal populations, if there are emerging clones with ertapenem resistance and if a resistance mechanism can be identified being responsible for the observed results [EURL-AR, personal communication].

6.3 Salmonella

DANMAP focuses on resistance in *Salmonella* Typhimurium and the related monophasic variants, as these serotypes are present in clinical human isolates and in isolates from food-producing animals, especially in pigs. Clonal dissemination plays an important role for the occurrence of antimicrobial resistance among *S. Typhimurium*. The global dissemination of genomic

islands conferring resistance to ampicillin, sulfamethoxazole and tetracycline (the ASuT multidrug-resistance profile) among *S. Typhimurium* continues to contribute to a high level of multidrug-resistance among isolates from humans and animals. In Denmark, *S. Typhimurium* from humans and food-producing animals, and in human isolates the monophasic variants, often carry ASuT resistance. The public health relevance of ASuT multidrug-resistance is of less direct importance than resistance to antimicrobials more commonly used in human medicine for salmonellosis treatment, such as macrolides, fluoroquinolones and cephalosporins. In DANMAP, *S. Typhimurium* includes the monophasic variants with antigenic formula *S.* 4, [5],12:i:-, unless otherwise stated.

6.3.1 Resistance in *S. Typhimurium*

In 2022, a total of 190 human isolates of *S. Typhimurium* were susceptibility tested, including 96 *S. Typhimurium* and 94 monophasic variants. The monophasic isolates were dominated by sequence type (ST) 34 (91 isolates) and *S. Typhimurium* variants were dominated by ST19 (53) and ST36 (35). Forty-two isolates were associated with six outbreaks. The largest outbreak encompassed 14 isolates, and 20 and 22 of the outbreak related isolates were di- and monophasic, respectively. Thirty isolates were from travel-associated cases.

Forty-eight isolates from Danish pork were recovered and susceptibility tested in 2022, including 15 *S. Typhimurium* isolates and 33 monophasic variants.

The resistance data for *S. Typhimurium* for a panel of 15 antimicrobials are presented in Table 6.2 human isolates and for domestic pork. The resistance from human isolates is also presented separately for *S. Typhimurium* and monophasic variants.

Ampicillin-, sulfamethoxazole- and tetracycline resistance are common both in isolates from humans and meat. As in previous years, occurrence of resistance continued to be overall higher in isolates from pork than in isolates from humans. Accordingly, the level of fully-sensitive human isolates was higher, 39%, compared to 10% in isolates from Danish pork. Notably, the percentage of fully-sensitive isolates has decreased in *S. Typhimurium* from both humans and pork, compared to 2021 (Figure 6.3).

Figure 6.3 presents the relative distribution in percent of AMR profiles for *S. Typhimurium* from human domestically-acquired and travel-related cases and from domestic pork. The majority of ASuT-resistant isolates from humans were monophasic ST34, which encompassed 52 out of the 53 isolates that exclusively exhibited resistance towards ASuT.

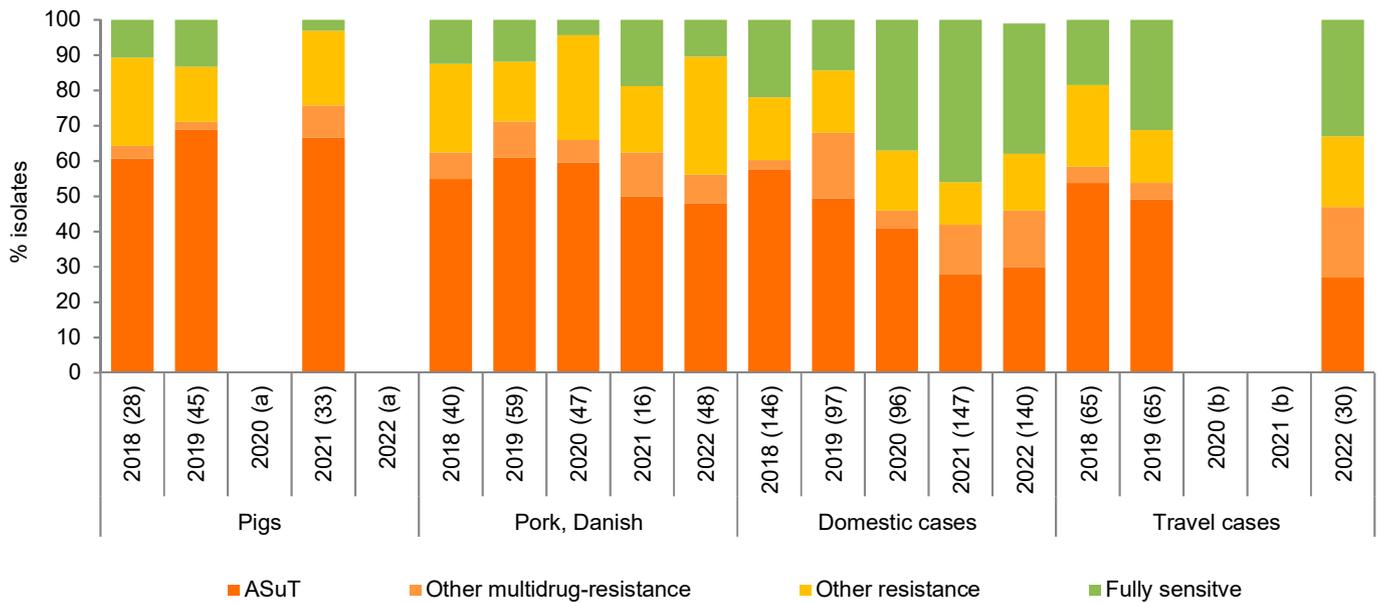
Table 6.2 Resistance (%) in *Salmonella Typhimurium* isolates from domestic pork and humans, Denmark

DANMAP 2022

| | Pork | | Human | | | | <i>S. Typhimurium</i> | <i>S. Typhimurium</i> monophasic |
|---------------------|--------|-----------------------|------------------------|----------------|-------|----|-----------------------|----------------------------------|
| | Danish | Domestically acquired | Travel abroad reported | Unknown origin | Total | | | |
| Antimicrobial agent | % | % | % | % | % | % | % | |
| Amikacin | 0 | 2 | 7 | 0 | 3 | 1 | 4 | |
| Ampicillin | 79 | 51 | 53 | 35 | 50 | 15 | 86 | |
| Azithromycin | 4 | 0 | 3 | 0 | 1 | 1 | 0 | |
| Cefotaxime | 0 | 1 | 3 | 0 | 1 | 0 | 2 | |
| Ceftazidime | 0 | 1 | 3 | 0 | 1 | 0 | 2 | |
| Chloramphenicol | 13 | 6 | 13 | 5 | 7 | 10 | 4 | |
| Ciprofloxacin | 0 | 4 | 20 | 0 | 6 | 8 | 3 | |
| Colistin | 0 | 1 | 7 | 0 | 2 | 2 | 1 | |
| Gentamicin | 8 | 1 | 3 | 0 | 1 | 0 | 2 | |
| Meropenem | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Nalidixic acid | 0 | 4 | 13 | 0 | 5 | 6 | 3 | |
| Sulfamethoxazole | 81 | 54 | 47 | 35 | 51 | 19 | 84 | |
| Tetracycline | 56 | 55 | 50 | 25 | 51 | 15 | 88 | |
| Tigecycline | 2 | 1 | 0 | 0 | 1 | 2 | 0 | |
| Trimethoprim | 25 | 11 | 7 | 5 | 9 | 4 | 15 | |
| Fully sensitive (%) | 10 | 37 | 33 | 65 | 39 | 74 | 4 | |
| Number of isolates | 48 | 140 | 30 | 20 | 190 | 96 | 94 | |

Includes isolates verified as monophasic variants of *S. Typhimurium* with antigenic formula *s.* 4,[5],12:i:-. Isolates of Danish pork were recovered from carcass swabs collected at slaughter. An isolate is categorised as domestically-acquired if the patient did not travel outside Denmark one week prior to the onset of disease. An isolate is considered fully-sensitive if susceptible to all antimicrobial agents included in the test panel (Chapter 10, Table 10.3)

Figure 6.3 Relative distribution (%) of multidrug-resistant, resistant and fully-sensitive *S. Typhimurium* from pigs, domestic pork and human cases, Denmark DANMAP 2022



Number of isolates included each year is presented in parentheses. Includes isolates verified as monophasic variants of *S. Typhimurium* with antigenic formula S. 4,[5],12:i:-. An isolate is considered fully-sensitive if susceptible to all antimicrobial agents included in the test panel, and multidrug-resistant if resistant to three or more of all antimicrobial classes included in the test panel (See Chapter 10, Table 10.3). ASuT are multidrug-resistant isolates resistant to ampicillin, sulfamethoxazole and tetracycline. Most ASuT human isolates correspond to monophasic variants of *S. Typhimurium*.

a) No data; b) Distribution not shown due to low number of isolates (N<15)

Most of the *S. Typhimurium* isolates recovered from domestic pork were resistant to several antimicrobials, and 56% were multidrug-resistant (48% of which were ASuT). Only 10% of the Danish pork isolates were fully-sensitive to all tested antimicrobials in 2022, which represents a decrease by 5% compared to 2021. Over the past five years, the resistance patterns among *S. Typhimurium* from Danish pork have fluctuated, and no obvious trends have been detected. However, since 2020 the occurrence of ASuT resistance has gradually decreased, at the exchange of an increase in occurrence of other multidrug-resistance and other resistance profiles (Figure 6.3).

Fluoroquinolones may be used for treatment of human *Salmonella* infections and resistance is monitored using ciprofloxacin. Ciprofloxacin resistance was observed in 4% of the isolates from domestically-acquired infections and in 20% of the isolates from travel-associated cases. Historically, ciprofloxacin resistance has predominantly been observed in isolates from travel-associated cases. During the last ten years, ciprofloxacin resistance in *S. Typhimurium* from Danish pork has been rare. In 2022, ciprofloxacin resistance was not found among isolates from pork (Figure 6.4).

Since 2014, macrolide resistance in *Salmonella* has been monitored using azithromycin, which is used for treatment of human *Salmonella* infections in Denmark. Resistance to azithromycin in *S. Typhimurium* has been low in human isolates and in 2022 it was only found in one isolate (1%). In 2021, azithromycin resistance was detected in a higher than usual

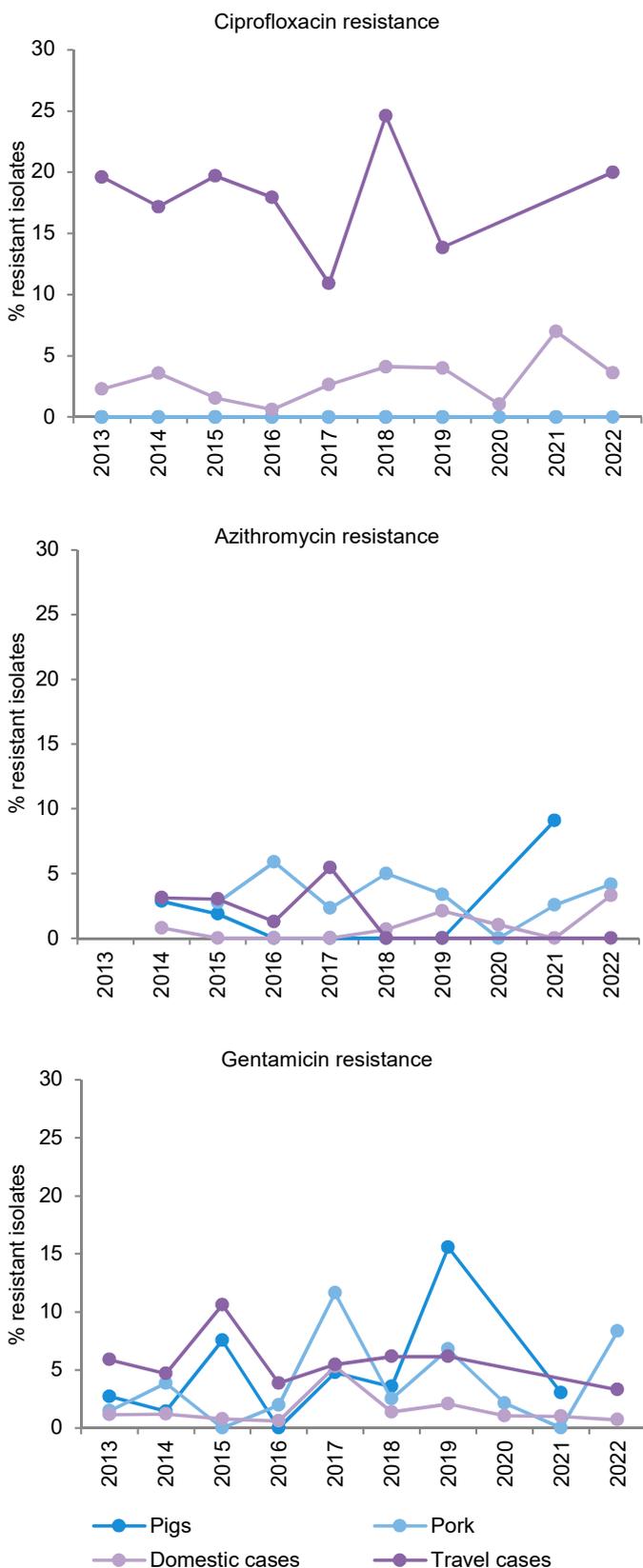
percentage of isolates from pigs. In 2022, *S. Typhimurium* data was not available from pigs, and the results from domestic pork showed an occurrence of 4%, similar to what has been observed in previous years (Figure 6.4).

The levels of gentamicin resistance have been low and stable over the last years, and in 2022 two human isolates (1%) were resistant towards gentamicin. After a decrease in 2020 and 2021 in the occurrence of resistance to gentamicin in isolates from pork, in 2022 it increased to a level (8%) similar to 2019 (Figure 6.4).

Among human isolates, the level of resistance towards 3rd generation cephalosporins was low, and the combination of cefotaxime and ceftazidime resistance was only found in two isolates. Meropenem resistance was not observed among human isolates. As in the previous years, none of the *S. Typhimurium* isolates from domestic pork were resistant to 3rd generation cephalosporins or to meropenem (Table 6.2).

Resistance to tigecycline and colistin in *S. Typhimurium* are rare in Denmark. In 2022, two human isolates were recorded as colistin-resistant, and as in the previous years, no colistin resistance was found among pork isolates. Resistance towards tigecycline was observed in two human isolates and in 2% of the pork isolates. Resistance to amikacin, the antimicrobial that was introduced in the test panel in 2021, was observed in 3% of the human *S. Typhimurium* isolates in 2022 and thus at the same level as in 2021. All isolates from pork were sensitive towards amikacin (Table 6.2).

Figure 6.4 Ciprofloxacin, azithromycin and gentamicin resistance (%) among *S. Typhimurium* from pigs, domestic pork and human cases, Denmark DANMAP 2022



Includes isolates verified as monophasic variants of *S. Typhimurium* with antigenic formula s. 4,[5],12:i:-. An isolate is categorised as domestically-acquired if the patient did not travel outside Denmark one week prior to the onset of disease. Due to the low number of isolates (N<15), travel-associated cases are not shown separately for 2020 and 2021. No data available for pigs in 2020 and 2022

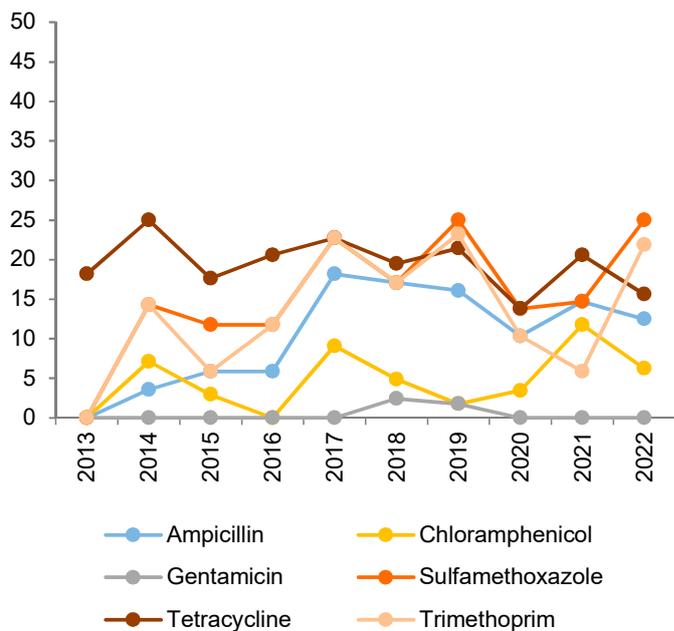
The occurrence of resistance to tetracycline in isolates from Danish pork seems to follow a decreasing trend, having decreased from 77% in 2020 to 67% in 2021 and 56% in 2022. The steady reduction in the use of tetracycline in pig production observed since 2014 (see Chapter 4, Table 4.1) may be one of the explanations for this observation.

6.3.2 Resistance in other *Salmonella* serotypes

Among samples from domestic pork, next to *S. Typhimurium*, the most common serotype detected was *S. Derby*, which was recovered from 32 samples in 2022.

The occurrence of resistance in *S. Derby* is generally lower than in *S. Typhimurium*, and in 2022, 69% of the *S. Derby* isolates from pork were susceptible to all tested antimicrobials, which was similar to the level observed in 2021. In 2022, compared to the previous year, resistance to sulfamethoxazole and trimethoprim increased, while resistance to ampicillin, tetracycline and chloramphenicol decreased (Figure 6.5). Resistance to critically important antimicrobials remained rare in 2022 in *S. Derby* isolates from domestic pork, with only one isolate resistant to azithromycin, and one isolate resistant to tigecycline. *S. Derby* isolates from domestic pork were not resistant to amikacin, 3rd and 4th generation cephalosporins, colistin, gentamicin, meropenem or fluoroquinolones (ciprofloxacin and nalidixic acid).

Figure 6.5 Resistance (%) among *Salmonella* Derby from domestic pork, Denmark DANMAP 2022



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Textbox 6.1

Detection of resistance genes and point mutations in *Salmonella* and *Campylobacter* using whole genome sequencing

Whole genome sequencing (WGS) is an integrated part of the surveillance of human *Campylobacter* and *Salmonella* infections in Denmark. The genome sequences are used to elucidate the genetic relationship between clones that prevail in Denmark in order to identify potential outbreaks, and the data is further used to identify genes and/or point mutations (genetic resistance markers) that confer antimicrobial resistance.

Resistance genotypes are established by WGS. DNA is extracted from the isolates, sequenced using the Illumina platform, and the quality of the sequence data is reviewed using the BIFROST QC pipeline (<https://github.com/ssi-dk/bifrost>). The genomes are assembled using SKESA (<https://github.com/ncbi/SKESA>) and the assemblies are analyzed with AMRFinderPlus (<https://github.com/ncbi/amr/wiki>), Software version: 3.11.18, Database version: 2023-08-08.2, with settings coverage 0.5, identity 0.9, for the in-silico detection of acquired resistance genes and point mutations.

Resistance genes and point mutations are widespread in Danish human clinical isolates in both *Campylobacter* and *Salmonella*. Many genetic resistance markers confer well defined phenotypic resistance and in this textbox, unless otherwise stated, the presence of the specified genetic markers usually confers phenotypic resistance.

The prevalence of genetic resistance markers for selected classes of antimicrobials are presented in Figure 1 for 649 clinical isolates of *C. jejuni* and 58 isolates of *C. coli*. Genetic resistance markers are common in both species but the frequency of resistance markers in *C. coli* is generally higher than the frequency in *C. jejuni*.

Genes conferring resistance towards tetracycline are present in 29% of *C. jejuni* and 55% of *C. coli* isolates. Fluoroquinolone resistance is usually associated with point mutations in the *gyrA* gene and 58% of *C. jejuni* and 69% of *C. coli* carry genetic markers that typically confer fluoroquinolone resistance. Aminoglycoside resistance markers are frequently identified in *C. coli* (36%) and less frequently in *C. jejuni* (6%), but the majority of the identified genetic aminoglycoside resistance markers are not conferring resistance towards gentamicin. Beta-lactam resistance markers are common in both species, but the clinical significance of many of the resistance genes remains to be resolved. This also applies to the genetic basis for ertapenem resistance. Resistance markers for macrolide resistance are also common in both species, and can be mediated by both genes and point mutations. However, the presence of the genetic macrolide markers rarely confers resistance towards erythromycin. Chloramphenicol resistance markers are rarely identified in both species.

The prevalence of genetic resistance markers for selected classes of antimicrobials are presented in Figure 2 for 782 clinical isolates of *Salmonella* from 2022. The isolates represent 101 monophasic *S. Typhimurium*, 101 *S. Typhimurium*, 246 *S. Enteritidis* and 334 isolates belonging to other serotypes.

Genetic resistance markers are common in all serotypes. It is prominent that the levels of genetic resistance markers for resistance to aminoglycosides, beta-lactams resistance, sulfonamides and tetracyclines are particularly high in monophasic *S. Typhimurium* reflecting phenotypic resistance towards ampicillin, streptomycin, sulfonamide, and tetracycline.

A number of different genetic resistance markers for gentamicin were found in seven isolates (1%). Genes conferring resistance towards neomycin, *aph(3')-Ia*, were found in 2% of all isolates and in 11% of monophasic *S. Typhimurium* isolates.

Among the genetic resistance markers for beta-lactam antimicrobials, *blaTEM-1* (ampicillin resistance) was identified in 14% of the isolates and it was harbored by 82% of the monophasic *S. Typhimurium* variants. Genes associated with extended spectrum beta-lactamase production were found in three isolates and genes conferring resistance towards carbapenems were not identified.

Genetic resistance markers for azithromycin resistance were found in five isolates (<1%) and included both isolates with genes *mph(A)* and isolates with point mutations in the *acrB* gene.

continued ... Textbox 6.1

Figure 1 Prevalence of resistance genes and/or point mutations conferring resistance to selected antimicrobial classes in 649 clinical human isolates of *C. jejuni* and in 58 isolates of *C. coli*, Denmark, 2022 DANMAP 2022

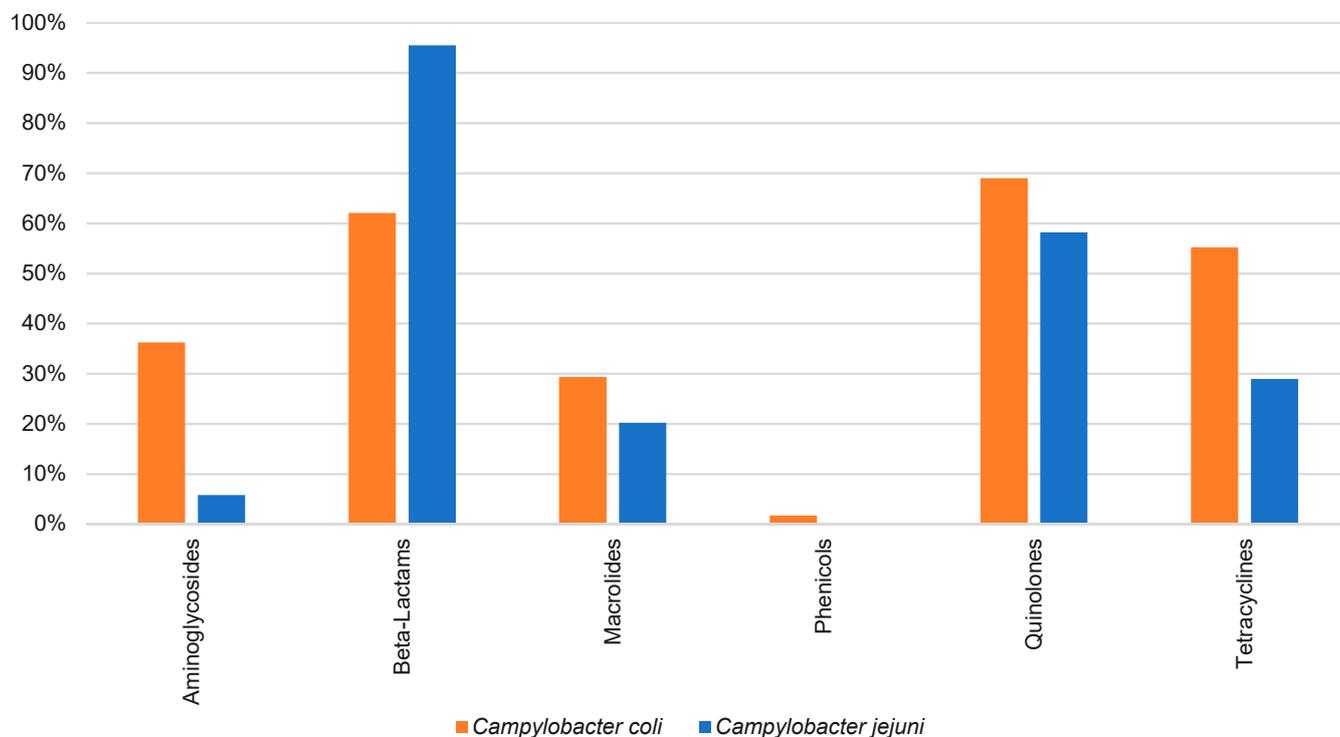
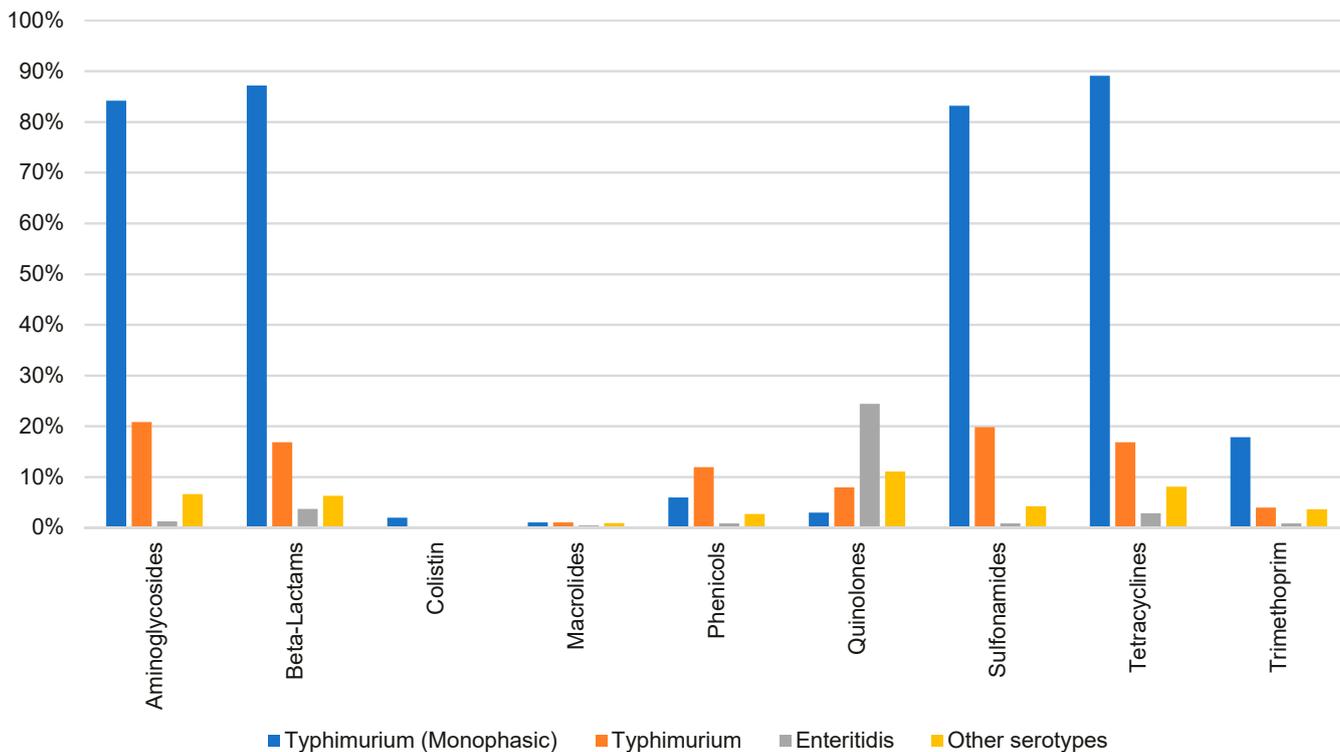


Figure 2 Prevalence of resistance genes and/or point mutation conferring resistance to selected antimicrobial classes in 782 clinical human isolates of *Salmonella*, Denmark, 2022 DANMAP 2022



Salmonella isolates included 101 monophasic *S. Typhimurium*, 101 *S. Typhimurium*, 246 *S. Enteritidis*, and 334 isolates belonging to other serotypes

Resistance markers conferring resistance towards fluoroquinolones were found in 14% of the isolates in total and in 24% of *S. Enteritidis* isolates. Resistance towards fluoroquinolones was mediated by point mutations in 10% of the isolates and by *qnr* genes in 4% of the isolates.

Perspectives

WGS is a valuable tool for surveillance of AMR in *Campylobacter* and *Salmonella* in human clinical isolates. In many cases it is possible to predict the phenotypic susceptibility of isolates based on WGS. More WGS-based data will be presented in the coming DANMAP reports, both for isolates recovered from humans and from healthy and sick animals. This will eventually allow to monitor the spread of genetic resistance markers, and combinations of markers along the farm to fork chain.

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