6

RESISTANCE IN ZOONOTIC BACTERIA

6. Resistance in zoonotic bacteria

Highlights: In 2024, resistance in ***Campylobacter jejuni*** isolates from humans remained lower in domestically acquired cases (ciprofloxacin 44%, tetracycline 22%) than in travel-associated cases (ciprofloxacin 85%, tetracycline 62%), but higher than in isolates recovered from broilers (ciprofloxacin 29%, tetracycline 17%) and cattle (ciprofloxacin 26%, tetracycline 1%). This is similar to previous years, albeit with some fluctuations. The proportion of fully susceptible *C. jejuni* has remained stable over time (69% in broilers, 73% in cattle and 46% in human cases for 2024). Erythromycin resistance remained rare in *C. jejuni* from cattle and was not observed in isolates from broilers and humans.

Campylobacter coli showed higher levels of resistance in human and broiler isolates compared to *C. jejuni*, with particularly high levels of resistance to ciprofloxacin (83% in travel-related cases, 66% in domestic cases and 54% in broilers) and tetracycline (75% in travel-related human cases, 72% in domestic human cases and 58% in broilers). Resistance to erythromycin was observed in 14% of human isolates and in 17% of isolates from broilers. This is an important finding as macrolides are used for the treatment of human patients in severe cases.

Phenotypic resistance in ***Salmonella* Typhimurium** and **monophasic *S*. Typhimurium** isolates remained overall higher in domestic pork than in humans. In addition to results from phenotypic resistance testing, DANMAP 2024 presents results from whole-genome sequencing of all isolates, representing the two *Salmonella* serovars, isolated the most between 2020 and 2024.

In 2024, two large **human outbreaks** caused by *S.* Typhimurium and monophasic *S.* Typhimurium affected the overall levels of phenotypic resistance in human isolates. The monophasic outbreak was caused by an atypical fully susceptible clone. In all 823 human isolates tested, resistance to third-generation cephalosporins remained low, and no resistance to meropenem was observed. Six human isolates (1%) were simultaneously resistant to azithromycin, third-generation cephalosporins and ciprofloxacin. Genetic determinants conferring resistance to quinolones, azithromycin, gentamicin, and third-generation cephalosporins were detected sporadically from 2020 to 2024. The yearly occurrence of commonly found resistance determinants, *blaCARB-2*, *blaTEM-1*, *floR*, *sul1*, *sul2*, *tet(A)*, *tet(B)*, *tet(G)*, was influenced by outbreak-associated clones.

Among *Salmonella* **isolates from pork**, the level of full susceptibility in *S.* Typhimurium and monophasic *S.* Typhimurium increased compared to 2023. Phenotypic ampicillin-, sulfamethoxazole-, and tetracycline resistance (ASuT) remained common in monophasic S. Typhimurium isolates and the most common genetic profile conferring ASuT resistance was reported. Phenotypic fluoroquinolone (ciprofloxacin) resistance remained undetected. Unlike in 2023, phenotypic azithromycin resistance was detected in two monophasic *S.* Typhimurium isolates from pork. The occurrence of phenotypic resistance to gentamicin remained similar to that in 2022 and 2023. Genes conferring resistance to amikacin and gentamicin were detected in monophasic *S.* Typhimurium, and the *aac(3)-IVa* gene, conferring resistance to gentamicin and apramycin, was found in three *S.* Typhimurium isolates.

6.1 Introduction

6.1.1 Introduction to resistance in zoonotic bacteria

Zoonoses are infectious diseases transmitted between animals and humans through direct contact or indirectly via contaminated food, water or environment. In DANMAP, information on antimicrobial resistance (AMR) in zoonotic bacteria is collected from the national programme for monitoring and control on zoonoses. For more information see the Annual Report on Zoonoses in Denmark 2024 [www.food.dtu.dk].

In humans, AMR is monitored in clinical *Salmonella* isolates. For *Campylobacter jejuni* and *C. coli*, a geographically stratified selection of clinical isolates is tested. 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.

In Denmark, antimicrobials are generally not recommended for treating human patients with diarrhoea apart from prolonged or severely ill cases. When necessary, macrolides (azithromycin) may be used for *Campylobacter* infections. In cases of prolonged or recurrent *Salmonella* infection, treatment with ciprofloxacin or trimethoprim-sulfamethoxazole may be considered, based on antimicrobial susceptibility testing. Information regarding concrete antimicrobial usage in diarrheal patients on patient level is currently not available.

In animals, *Salmonella* isolates were obtained from carcasses of healthy pigs at slaughter, while *Campylobacter* isolates were obtained from caecal samples from healthy broilers and cattle at slaughter. Antimicrobial susceptibility testing of *Campylobacter* and *Salmonella* from animals and meat is done in accordance with the Commission Implementing Decision 2020/1729/EU of 17 November 2020 (see Chapter 10 for further details).

In Danish food-producing animals, macrolides are often used to treat infections, particularly in pigs. Fluoroquinolones and 3rd and 4th generation cephalosporins are not used in production animals. The use of antimicrobials in the Danish poultry sector is low and limited to a few antimicrobial classes, primarily tetracyclines (see Chapter 4, Table 4.1).

6.2 *Campylobacter* spp.

A total of 200 human isolates of *C. jejuni* (158 domestic, 39 travel-associated, 3 unknown) and 44 isolates of *C. coli* (29 domestic, 12 travel-associated, 3 unknown) were tested for antimicrobial susceptibility.

In animals, all *C. jejuni* isolates recovered from broilers (202) and cattle (93), as well as all *C. coli* isolates from broilers (52) and cattle (4), were also tested for antimicrobial susceptibility. Due to the low number of isolates recovered, resistance data for *C. coli* from cattle are not reported.

6.2.1 Resistance in *Campylobacter jejuni*

The resistance levels recorded in *C. jejuni* isolates from humans, Danish broilers and cattle at slaughter in 2024 are shown in Table 6.1. The ten-year trends in resistance to selected antimicrobials are shown in Figure 6.1.
In 2024, 69% of *C. jejuni* from broilers, 73% from cattle and 46% from human cases (54% from domestically acquired and 13% from travel-related cases) were fully susceptible to all tested antimicrobials. The percentage of fully susceptible *C. jejuni* isolates from broilers, cattle and human cases has remained stable over the past decade (Figure 6.1).

Overall, resistance levels in *C. jejuni* from human isolates remained stable over the last ten years, with notably higher resistance levels in isolates from travel-associated cases compared to domestic. Human isolates also have generally higher levels of resistance than isolates from broilers and cattle.

Resistance to ciprofloxacin and tetracycline remained common in *C. jejuni* from both humans and food-producing animals, except in cattle, where tetracycline resistance was only observed in one isolate.

As in previous years, combined resistance to ciprofloxacin and tetracycline in *C. jejuni* isolates was common; 27% from human cases (54% in travel-related and 20% in domestically acquired cases) and 16% from broilers (Table 6.1 and Figure 6.1). Over the last 10 years, the prevalence of isolates with this resistance profile has fluctuated slightly in domestic human cases and in broilers, but remained stable for travel-related cases and in cattle (Figure 6.1).

Macrolide (erythromycin) resistance in *C. jejuni* is rare. In 2024, it was not observed in human and broiler isolates and was found in only one isolate from cattle. Resistance to chloramphenicol and gentamicin is also rare and in 2024, no resistance to chloramphenicol was detected. Similarly, no gentamicin resistance was recorded in isolates from humans and cattle, but two resistant *C. jejuni* isolates from broilers were reported (1%).

Ertapenem resistance has been monitored in *Campylobacter* since 2021. However, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has not proposed an epidemiological cut-off (ECOFF) or clinical breakpoint for ertapenem resistance, which is crucial for correctly identifying whether an isolate is susceptible or resistant. Therefore, the clinical relevance of human strains resistant to ertapenem is unclear. The EU Reference Laboratory for AMR (EURL-AMR) and the European Food Safety Authority (EFSA) have established a provisional ECOFF of 0.5 mg/L (EFSA, 2024), adopted in DANMAP.

Among *C. jejuni* isolates from broilers, 7% were resistant to ertapenem - an increase of 5% from the previous year. No ertapenem resistance was found in cattle isolates.

Table 6.1 Resistance (%) in *Campylobacter jejuni* isolates from broilers, cattle and human cases, Denmark, 2024 DANMAP 2024

	Broilers	Cattle	Human	
	Danish	Danish	Domestically acquired	Travel abroad reported
Antimicrobial agent	%	%	%	%
Chloramphenicol	0	0	0	0
Ciprofloxacin	29	26	44	85
Ertapenem	7	0	4	5
Erythromycin	0	1	0	0
Gentamicin	1	0	0	0
Tetracycline	17	1	22	62
CIP-TET	16	0	20	54
Fully susceptible	69	73	54	13
Number of isolates	202	93	158	39

An isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease
The threshold for resistance to ertapenem in *Campylobacter* has not been validated by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)
CIP TET: all isolates with both ciprofloxacin and tetracycline resistance. Fully susceptible: isolates sensitive to all antimicrobial agents included in the test panel (See Chapter 10, Table 10.3)

6.2.2 Resistance in *Campylobacter coli*

The resistance data for *C. coli* isolates from humans and Danish broilers at slaughter in 2024 are presented in Table 6.2.

Historically, AMR in *C. coli* from these sources has not been a focus of DANMAP, limiting longitudinal comparisons, particularly for broiler isolates. However, comparisons with 2023 data are possible for human isolates. The data from humans indicate high levels of resistance in *C. coli*. In travel-related cases resistance to ciprofloxacin was 83% and to tetracycline 75%, in domestic cases it was 75% to ciprofloxacin and 72% to tetracycline. The levels are comparable to those observed in 2023, the first year of reporting human data, and higher than the levels observed in human *C. jejuni* isolates.

Resistance in *C. coli* from broilers was also high, with 54% and 58% of the isolates resistant to ciprofloxacin and tetracycline, respectively.These resistance levels are higher than those observed in *C. jejuni* isolates from broilers and humans (Tables 6.1 and 6.2).

Resistance to erythromycin was observed in 14% of human isolates, and in 17% of isolates from broilers. This is important as macrolides are frequently used for the treatment of severely ill patients.

Gentamicin resistance was low, detected in only 8% of the travel-related and none of the domestic human cases, and it was not detected in broilers. Similarly, no resistance to chloramphenicol was observed in any of the human and broiler isolates.

While in 2023, no resistance to ertapenem was reported in *C. coli* from pigs, in 2024, it was commonly observed in isolates from broilers (37%).

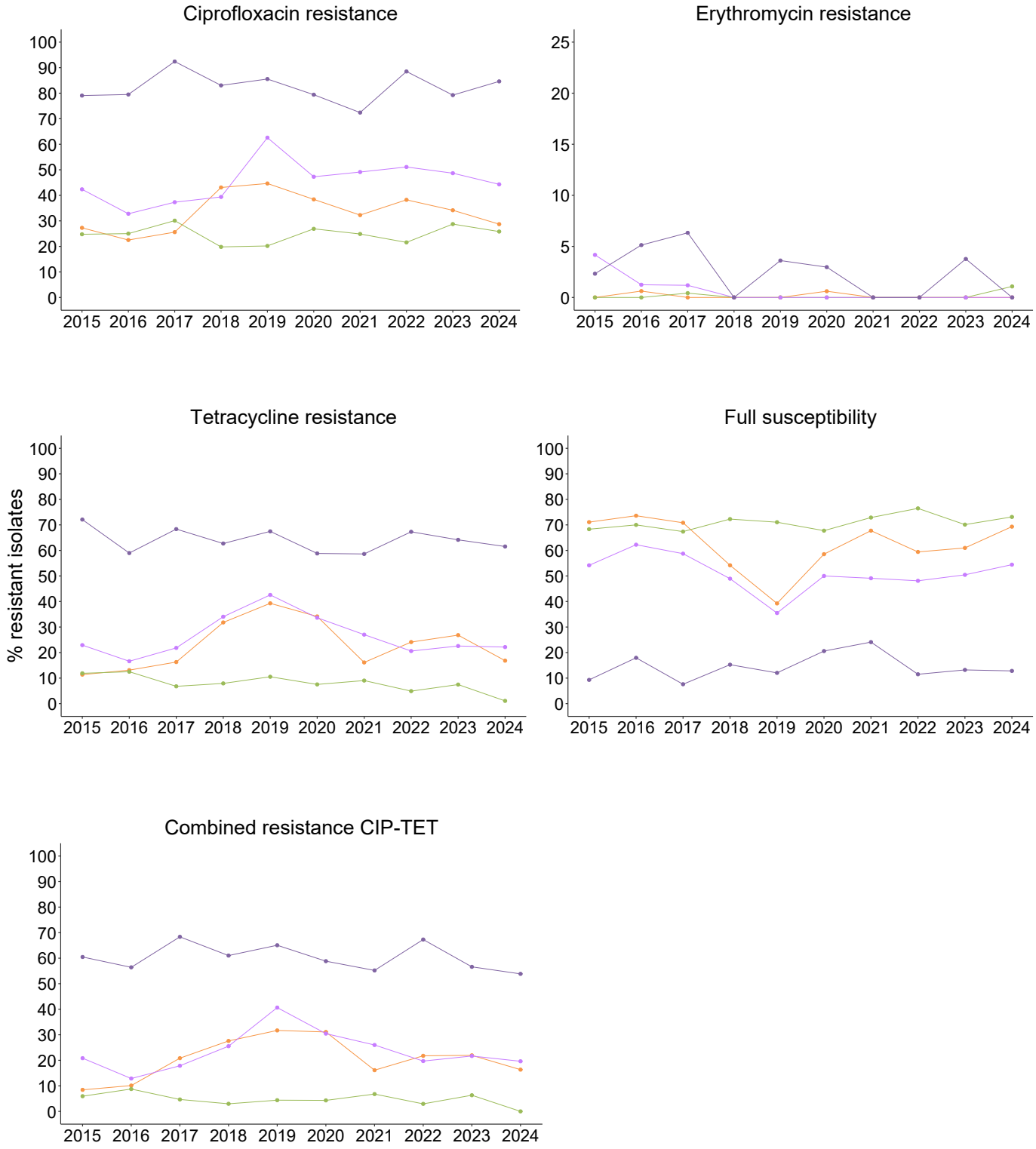
Overall, the comparably high levels of resistance in *C. coli* from human isolates and isolates from broilers point towards broilers being a probable source of infection with *C. coli* in humans.

Table 6.2 Resistance (%) in *Campylobacter coli* isolates from broilers and human cases, Denmark, 2024 DANMAP 2024

	Broilers	Human	
	Danish	Domestically acquired	Travel abroad reported
Antimicrobial agent	%	%	%
Chloramphenicol	0	0	0
Ciprofloxacin	54	66	83
Ertapenem	37	28	25
Erythromycin	17	7	25
Gentamicin	0	0	8
Tetracycline	58	72	75
Fully susceptible	21	14	8
Number of isolates	52	29	12

An isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. The threshold for resistance to ertapenem in *Campylobacter* has not been validated by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)

Figure 6.1 Resistance to ciprofloxacin, tetracycline, erythromycin, combined ciprofloxacin and tetracycline resistance and full susceptibility (%) among *Campylobacter jejuni* from broilers, cattle and human cases, Denmark, 2015-2024 DANMAP 2024



An isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease
CIP TET: all isolates with both ciprofloxacin and tetracycline resistance. Fully susceptible: isolates sensitive to all antimicrobial agents included in the test panel. All data shown result from a minimum of 15 tested isolates (see Chapter 10 for more details)

6.3 *Salmonella* spp.

In 2024, a total of 823 human clinical *Salmonella* spp. isolates, representing 102 different serotypes, were tested for antimicrobial susceptibility. The predominant serotypes were *S.* Typhimurium (178), monophasic *S.* Typhimurium with the antigenic formula 4,[5], 12:i:- (133), and *S.* Enteritidis (44). Except for *S.* Enteritidis, the tested isolates represented all clinical Danish isolates and included outbreak isolates.

Two large *Salmonella* outbreaks took place in 2024. One involved 70 cases of *S.* Typhimurium resistant to sulfamethoxazole and tetracycline, and the other involved 66 cases of fully susceptible monophasic *S.* Typhimurium. Two other outbreaks encompassed 22 fully susceptible *S.* Umbillo cases and 11 cases of *S.* Typhimurium, which were resistant to ampicillin, chloramphenicol, sulfamethoxazole and tetracycline.

The resistance data for *S.* Typhimurium and monophasic *S.* Typhimurium, and for other serotypes from humans from 2024 are presented in Tables 6.3 and 6.4, respectively.

Also in 2024, a total of 96 *Salmonella* spp. isolates from domestic pork were tested for antimicrobial susceptibility. The most common serotypes reported in pork were monophasic *S.* Typhimurium variant 4,[5], 12:i:- (40), *S.* Derby (28), and *S.* Typhimurium (15). The resistance data from domestic pork isolates are presented in Table 6.3 for *S.* Typhimurium and monophasic *S.* Typhimurium and in Figure 6.5 for *S.* Derby.

Table 6.3 Resistance (%) in *Salmonella* Typhimurium and monophasic *S.* Typhimurium isolates from domestic pork and humans, Denmark, 2024

DANMAP 2024

	Pork		Human					
	S. Typhimurium	Monophasic *S.* Typhimurium	*S.* Typhimurium			Monophasic *S.* Typhimurium		
	Danish	Danish	Domestically acquired	Travel abroad reported	Total	Domestically acquired	Travel abroad reported	Total
Antimicrobial agent	%	%	%	%	%	%	%	%
Amikacin	0	3	0	0	0	0	0	0
Ampicillin	80	80	12	22	13	39	81	46
Azithromycin	0	5	0	0	0	1	14	3
Cefotaxime	0	0	0	0	0	2	10	3
Ceftazidime	0	0	0	0	0	2	10	3
Chloramphenicol	47	5	10	22	12	6	29	8
Ciprofloxacin	0	0	2	26	6	2	33	7
Colistin	0	0	1	0	1	1	10	2
Gentamicin	20	8	0	0	0	1	5	2
Meropenem	0	0	0	0	0	0	0	0
Nalidixic acid	0	0	2	19	4	3	33	8
Sulfamethoxazole	73	85	69	44	62	35	71	41
Tetracycline	47	80	66	44	60	32	81	41
Tigecycline	0	3	10	0	8	7	5	7
Trimethoprim	47	25	2	19	4	8	38	12
Fully susceptible	7	10	27	52	34	55	10	46
Number of isolates	15	40	124	27	178	88	21	133

Results are shown separately for *S.* Typhimurium and monophasic *S.* Typhimurium with antigenic formula *S.* 4,[5],12:i:-. Isolates from 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. Total number of human cases includes travel cases and infections of unknown origin. An isolate is considered fully susceptible if sensitive to all antimicrobial agents included in the test panel (Chapter 10, Table 10.3)

6.3.1 Resistance in *S.* Typhimurium, monophasic *S.* Typhimurium and other selected *Salmonella* serovars

DANMAP has historically focused on phenotypic resistance in *S.* Typhimurium and the related monophasic variant 4,[5],12:i:-, as these serotypes are prevalent in clinical human isolates and isolates from food-producing animals and derived products.

The current report only presents available data from human clinical isolates and domestic pork isolates. The lack of availability of AMR results from isolates from a wider variety of sources limits the options for comparison of resistance levels in animals at slaughter, food, and humans. It is known that there are other sources of human salmonellosis infections than domestic pork, and clonal dissemination plays an important role in the occurrence of antimicrobial resistance among *S.* Typhimurium and monophasic *S.* Typhimurium.

The resistance levels recorded in *S.* Typhimurium and monophasic *S.* Typhimurium isolates from pigs and humans in 2024 are shown in Table 6.3. Figure 6.2 presents the relative distribution (%) of AMR profiles for *S.* Typhimurium and monophasic *S.* Typhimurium from pigs, domestic pork and human domestic- and travel-related cases from 2020 to 2024. The ten-year trends in resistance to selected antimicrobials among *S.* Typhimurium combined with monophasic *S.* Typhimurium from pigs, domestic pork and human cases are shown in Figure 6.3. In addition, Table 6.4 shows resistance levels in other *Salmonella* serovars from human cases in 2024, and Figure 6.5 shows trends of resistance among *S.* Derby isolates from domestic pork in 2015-2024.

Resistance in human isolates

The resistance profile of the human outbreak related isolates affected the levels of resistance reported in 2024 for monophasic *S.* Typhimurium and *S.* Typhimurium which makes it difficult to compare the levels of resistance with the previous years. However, if the outbreak related isolates are disregarded, the resistance levels and AMR profiles, are overall similar to the last five years.

Most of the human *Salmonella* spp. isolates, 58%, were susceptible to all antimicrobial tested. Resistance to third generation cephalosporins, (cefotaxime and ceftazidime) was recorded in 2% of the total of 823 clinical *Salmonella* spp. isolates from humans and azithromycin resistance was observed in 1% of the isolates. Ciprofloxacin resistance was observed in 6% of *S.* Typhimurium, 7% of monophasic *S.* Typhimurium, and in 15% of other serotypes (Tables 6.3 and 6.4). Same sentence is at the end of this paragraph. Generally, resistance to third-generation cephalosporins, azithromycin and ciprofloxacin were higher in isolates from travel-associated cases than from domestically acquired cases. Meropenem resistance was not recorded in 2024 in human isolates.

Six human isolates (1%) were simultaneously resistant to azithromycin, third generation cephalosporins and ciprofloxacin and thereby resistant towards antimicrobials that frequently are used for empirical treatment of human infections. The infections in the six cases were caused by monophasic *S.* Typhimurium (3), *S.* Muenster (1) and *S.* Saintpaul (2). Three of the six cases were associated with travel and three cases were acquired domestically.

The level of gentamicin resistance remained at a low level, with three isolates being resistant in 2024, two monophasic *S.* Typhimurium isolates and one *S.* Corvallis isolate (Table 6.3 and Figure 6.2).

Table 6.4 Resistance (%) in other *Salmonella* serovars from humans, Denmark, 2024 DANMAP 2024

	Other *Salmonella* serovars		
	Human		
	Domestically acquired	Travel abroad reported	Total
Antimicrobial agent	%	%	%
Amikacin	0	0	0
Ampicillin	5	12	8
Azithromycin	2	1	1
Cefotaxime	2	3	2
Ceftazidime	2	3	2
Chloramphenicol	4	4	4
Ciprofloxacin	8	22	15
Colistin	2	3	4
Gentamicin	0	0	0
Meropenem	0	0	0
Nalidixic acid	7	19	13
Sulfamethoxazole	5	7	6
Tetracycline	10	12	10
Tigecycline	10	8	10
Trimethoprim	4	3	4
Fully susceptible (%)	76	67	69
Number of isolates	183	233	512

Other *Salmonella* serovars exclude isolates verified as *S.* Typhimurium and 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. Total number of human cases includes travel cases and infections of unknown origin. An isolate susceptible to all antimicrobial agents included in the test panel is considered fully susceptible (Chapter 10, Table 10.3)

Polymyxin (colistin) resistance is mainly seen in *S.* Dublin and *S.* Enteritidis isolates. Both serotypes, in particular *S.* Dublin, are regarded as intrinsically resistant towards polymyxins.

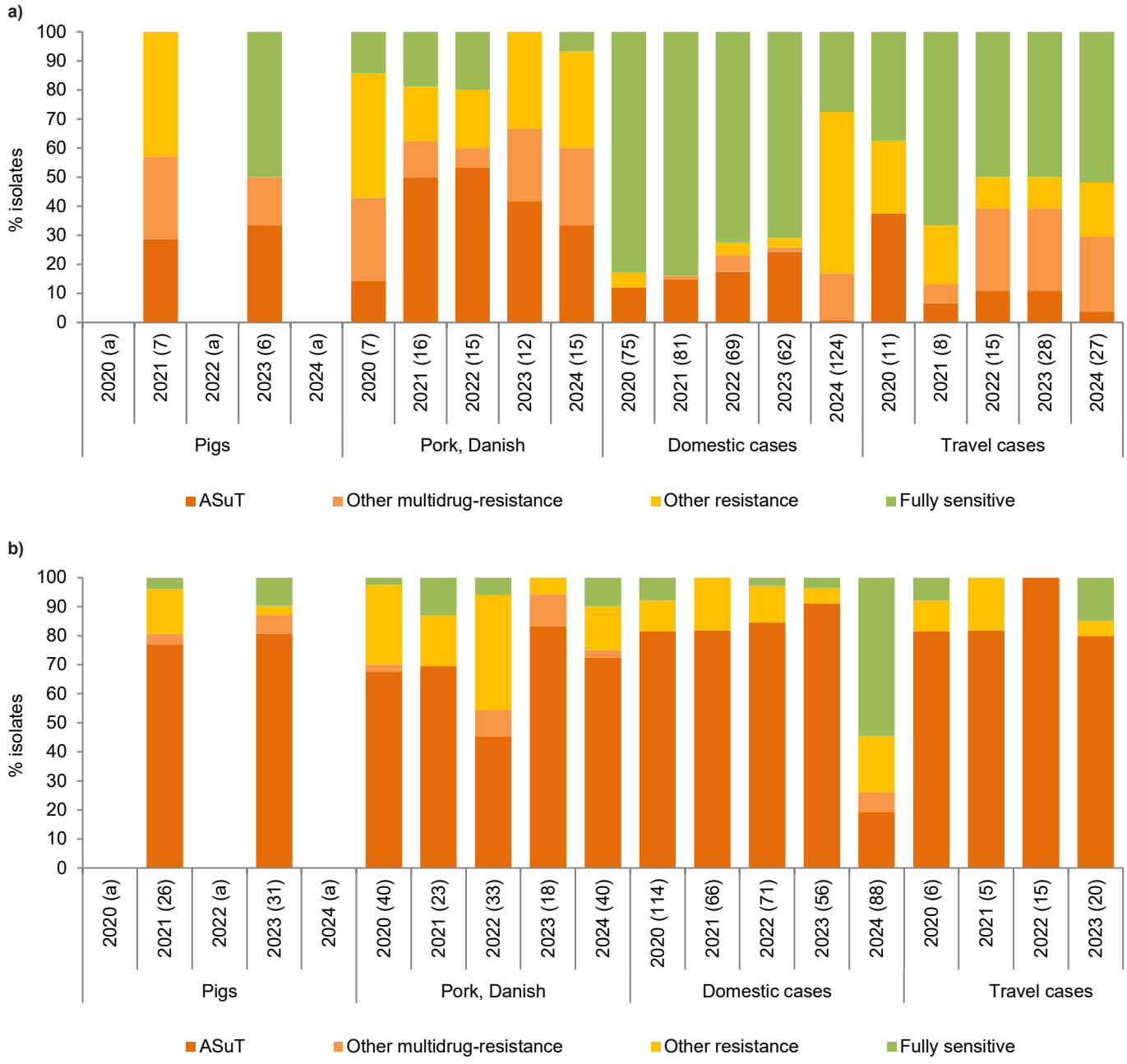
Resistance in pork isolates

As in previous years, the occurrence of resistance continued to be overall higher in isolates from domestic pork than in isolates from humans.

The level of full susceptibility in *S.* Typhimurium (7%) and monophasic *S.* Typhimurium (10%) from pork showed an increase when compared to 2023 (Figure 6.3A and B).

As in previous years, resistance to third generation cephalosporins, (cefotaxime and ceftazidime) was not observed in *S.* Typhimurium and monophasic *S.* Typhimurium isolates from domestic pork (Table 6.3).

Figure 6.2 Relative distributions (%) of AMR profiles among *Salmonella* Typhimurium (a) and monophasic *S.* Typhimurium (b) from pigs, pork and human cases, Denmark, 2020-2024
DANMAP 2024



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 susceptible if sensitive 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. Caution in data interpretation should be taken in years with small numbers (n <15) of isolates from domestic pork, pigs and travel-associated cases
(a) No data

The occurrence of resistance to tetracycline in monophasic *S.* Typhimurium from Danish pork remained high (80%) with similar levels as those found in 2023 (89%). Resistance to tetracycline in *S.* Typhimurium isolates was lower (47%).

Genomic islands conferring resistance to ampicillin, sulfamethoxazole and tetracycline (the ASuT multidrug-resistance profile) among monophasic *S.* Typhimurium contribute to a high level of multidrug-resistance (MDR) (Figure 6.2).

Most *S.* Typhimurium isolates recovered from domestic pork were resistant to several antimicrobials, with MDR levels reaching 60%. The ASuT MDR profile was found in the majority of the MDR *S.* Typhimurium isolates from pork (33%).

When looking at monophasic *S.* Typhimurium, MDR was found at higher levels, with 75% of isolates from domestic pork resistant to three or more antimicrobial classes and ASuT the most found MDR profile (73%).

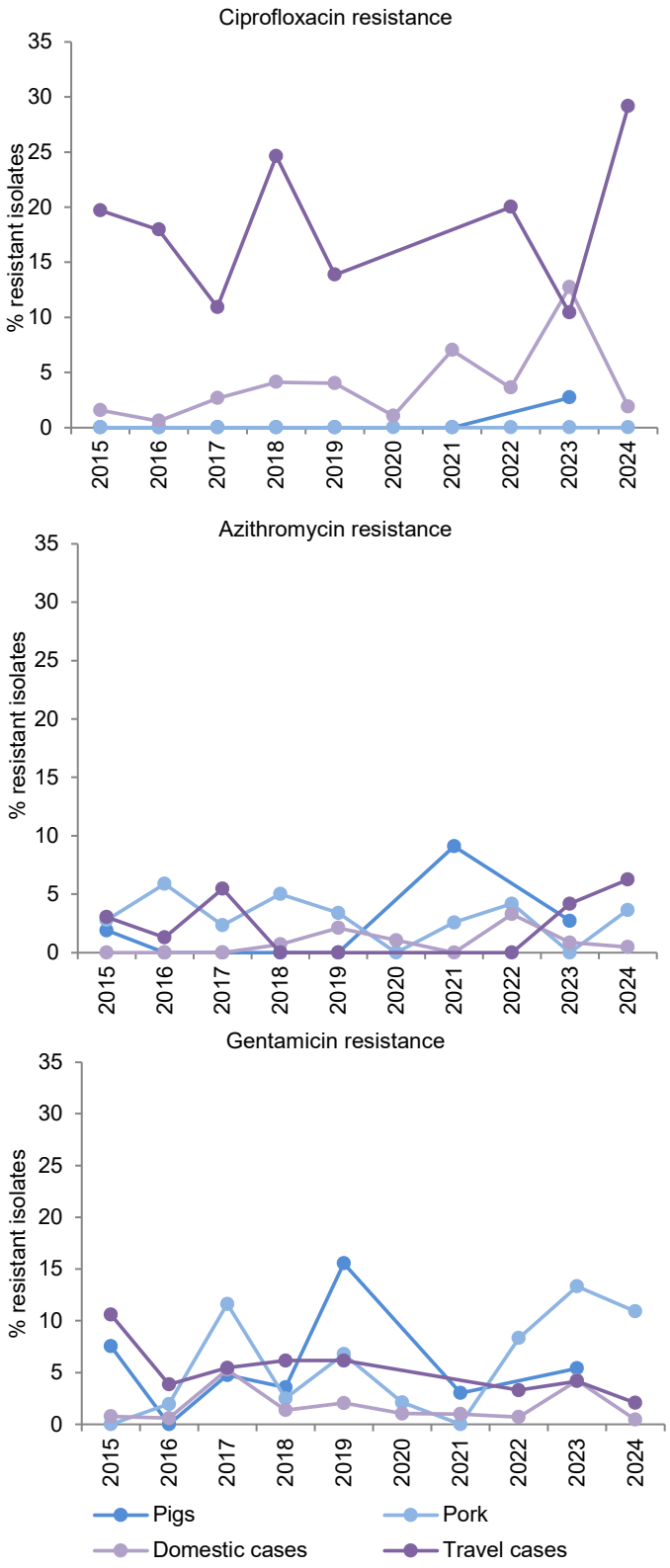
Analysing *S.* Typhimurium and monophasic *S.* Typhimurium separately reveals marked differences in their levels of MDR and especially ASuT. These are most evident in human isolates (Figure 6.2). However, caution in data interpretation should be taken in years when a small number of isolates were recovered from domestic pork, pigs and travel-associated cases.

Ciprofloxacin resistance has not been found among isolates from domestic pork since 2015 (Figure 6.3). Unlike in 2023, azithromycin resistance was detected in pork, with all resistant isolates belonging to the monophasic *S.* Typhimurium serotype (Figure 6.3).

In domestic pork, the occurrence of resistance to gentamicin (11%) remained similar to that in 2022 and 2023 (8% and 13%, respectively) (Figure 6.3).

Figure 6.3 Ciprofloxacin, azithromycin and gentamicin resistance (%) among *S.* Typhimurium and its monophasic variants from pigs, domestic pork and human cases, Denmark, 2015-2024

DANMAP 2024



Includes isolates of *S.* Typhimurium and monophasic *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, 2022 and 2024

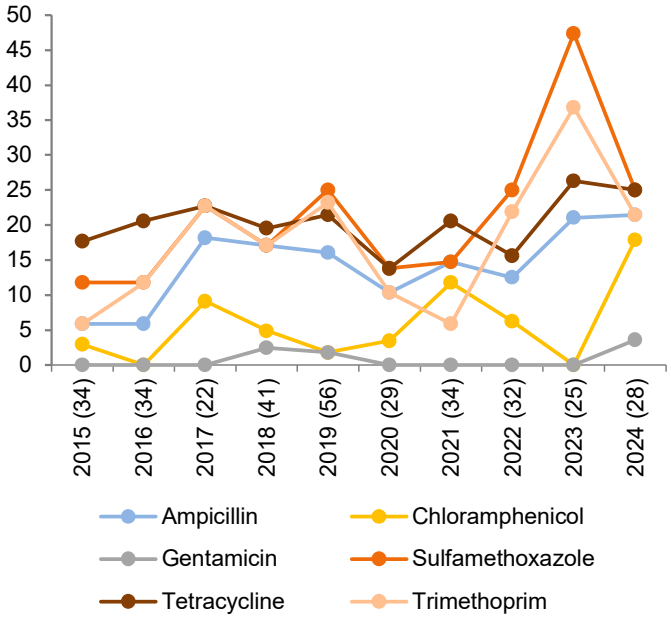
Resistance in other *Salmonella* serotypes from pork

S. Derby was the second most prevalent serotype in domestic pork, with 28 isolates recovered. The occurrence of resistance in *S.* Derby is generally lower than in *S.* Typhimurium and monophasic *S.* Typhimurium. In 2024, 64% of *S.* Derby isolates from domestic pork were fully susceptible to all tested antimicrobials. After a decrease in 2023 (to 52%), full susceptibility levels are again comparable to what was seen in 2022 (69%). After an increase in resistance to ampicillin, tetracycline, sulfamethoxazole, and trimethoprim in 2023, this year, resistance levels to ampicillin and tetracycline remained similar, while a decrease in sulfamethoxazole and trimethoprim was detected (Figure 6.5).

Resistance to critically important antimicrobials remained rare in 2024 in *S.* Derby isolates from domestic pork. Resistance to azithromycin and tigecycline was observed in 4% and 7% of pork isolates, respectively. Unlike the previous four years, resistance to gentamicin was detected (4%), but at a low level.

Additionally, *S.* Derby isolates from domestic pork showed no resistance to amikacin, 3rd generation cephalosporins, colistin, meropenem, or fluoroquinolones.

Figure 6.5 Resistance (%) among *Salmonella* Derby from domestic pork, Denmark, 2015-2024 DANMAP 2024



6.3.2 Genotypic resistance in *S.* Typhimurium and monophasic *S.* Typhimurium

Whole Genome Sequencing (WGS) can be used to complement antimicrobial susceptibility tests by identifying the genetic resistance mechanisms behind phenotypically expressed antimicrobial resistance. These mechanisms can be either induced by the specific genes or combination of genes, and/or point mutations. WGS makes it possible to investigate the dissemination of different genotypes across reservoirs.

S. Typhimurium and monophasic *S.* Typhimurium from domestic pork and human clinical isolates collected from 2020 to 2024 were investigated for genetic AMR determinants. A detailed description of the selection of isolates and methods used is included in Chapter 10.

In total, 466 *S.* Typhimurium isolates (379 domestic, 87 travel-associated) and 408 monophasic *S.* Typhimurium (341 domestic, 67 travel-associated) obtained from human cases in the period from 2020 to 2024, were included in the analysis of genetic AMR determinants. Overall, 61 and 76 unique genetic AMR determinants were identified in *S.* Typhimurium and monophasic *S.* Typhimurium isolates from humans, respectively.

All the sequenced *S.* Typhimurium (57) and monophasic *S.* Typhimurium (145) isolates from Danish pork collected from 2020 to 2024 in accordance with the EU rules for AMR monitoring were included.

The occurrence of selected AMR genes and point mutations (%) among *S.* Typhimurium and monophasic *S.* Typhimurium from domestic pork and human cases from 2020 to 2024 is presented in Figure 6.4. Only the most commonly occurring genetic AMR determinants conferring resistance to antimicrobials included in phenotypic testing (see Chapter 10, Table 10.3) are shown.

Resistance determinants in human *S.* Typhimurium isolates

In both domestic and travel-associated *S.* Typhimurium isolates from humans, *blaTEM-1*, *sul1*, *sul2* and *tet(A)* genes were the most frequently detected AMR determinants. Overall, the occurrence of these genes remained fairly stable over the years, albeit with some exceptions. In 2024, domestic isolates showed a marked increase in *sul2* and *tet(A)*, driven by an outbreak-associated *S.* Typhimurium clone with a specific AMR profile *aph(3")-Ib/aph(6)-Id/sul2/tet(A)*.

An increase of a few other genes (*floR*, *sul1*, *blaCARB-2*, and *tet(G)* was seen in domestic isolates in the period from 2022 to 2024 (Figure 6.4 A). In 2024, the increase was due to the outbreak-associated clone with a specific genetic profile *aadA2/blaCARB-2/floR/qacEdelta1/sul1/tet(G)* (n=11), and in 2022 and in 2023 due to a clone with a similar genetic profile *aadA2/blaCARB-2/floR/gyrA_D87N/qacEdelta1/sul1/tet(G)*.

An increase of *blaTEM-1* and *floR* observed in travel-associated isolates was not related to any specific *S.* Typhimurium clone (Figure 6.4a).

Resistance determinants to quinolones included five variants of the *qnr* gene (*qnrS1, qnrB19, qnrB2, qnrS13, qnrVC*), five different mutations in *gyrA* (D87N, S83Y, S83F, D87G, D87Y), and one mutation in *parC* (S80I). Over the five-year period, these determinants were detected infrequently, with only few (1-5) isolates identified per year.

The *gyrA* mutation D87N and *qnrS1* were dominating in domestic human isolates and in travel-associated isolates, respectively. *gyrA* mutations D87G, D87Y, S83F, *parC* mutation S80I, *qnrB2* and *qnrVC* were exclusively found in travel-associated isolates, whereas *gyrA* mutation S83Y was present only in domestic isolates. *qnrB19* and *qnrS13* were present in similar frequency.

Gentamicin resistance determinants, *aac(3)-IVa* and *aac(3)-IId* were detected in one domestic human isolate in 2023 and in one travel-associated isolate in 2024, respectively.

Azithromycin resistance determinants were detected in period from 2021 to 2023: *mph(A)* was detected in two travel-associated isolates, one in 2021 and one in 2022; and the *acrB* mutation R717 was detected in two travel-associated isolates in 2023.

One isolate from a domestic human case in 2021 carried *blaCMY-2* gene, conferring resistance to third-generation cephalosporins. Determinants conferring resistance to colistin and carbapenems were not detected.

Resistance determinants in pork *S.* Typhimurium isolates

Overall, in *S.* Typhimurium isolates from pork collected from 2020 to 2024, genes conferring resistance to beta-lactams (penicillins; *blaTEM-1*), sulfamethoxazole (*sul2*, *sul1* and *sul3*), tetracycline (*tet(A), tet(B)* and *tet(G)*), and trimethoprim (*dfrA12* and *dfrA1*) were the most commonly detected, matching with findings from phenotypical testing (Figure 6.4A). *aac(3)-IVa*, which confers resistance to aminoglycosides (gentamicin and apramycin), was first detected in 2022 and was found in three *S.* Typhimurium isolates in 2024 (Figure 6.4A).

The ASuT MDR genotypic profile was the most identified, present in 25 pork isolates. However, 16 different gene combinations were found, with *aph(3")-Ib/aph(6)-Id/blaTEM-1/sul2/tet(A)* found in five isolates, and *aph(3")-Ib/aph(6)-Id/blaTEM-1/sul2/tet(B)* and *aadA2/floR/blaCARB-2/sul1/tet(G)* in three isolates each.

Consistent also with the phenotypical findings, resistance genes conferring resistance towards carbapenems, 3rd, 4th and 5th generation cephalosporins, fluoroquinolones and polymyxins were not detected in *S.* Typhimurium from pork (Figure 6.5A and Table 6.3).

Resistance determinants in human monophasic *S.* Typhimurium isolates

blaTEM-1, *sul2*, and *tet(B)* were the most common genes detected in both domestic and travel-associated human isolates of monophasic *S.* Typhimurium over the five-year period in association with the genetic AMR profile *aph(3)-Ib/aph(6)-Id/blaTEM-1/sul2/tet(B)*. A combination of these genes is typically included in the epidemic monophasic *S.* Typhimurium (ST34) circulating among human cases in the EU. A notable decrease of these three genes was observed in domestic human isolates in 2024 due to the large outbreak caused by a fully susceptible monophasic *S.* Typhimurium clone.

A few AMR determinants showed higher occurrence in travel-associated human isolates than in the domestic isolates: *floR* conferring resistance to chloramphenicol and florfenicol; *qnrS1* and *qnrB19* conferring resistance to quinolones; and *tet(A)* conferring resistance to tetracycline (Figure 6.4b).

Seven unique quinolone resistance determinants distributed among 32 human isolates in the period from 2020 to 2024 were detected. *qnrS1* and *qnrB19* were the most frequent determinants with *qnrS1* detected in five domestic and eight travel-associated isolates, and *qnrB19* in six domestic and four travel-associated isolates. Other quinolone resistance determinants, *gyrA* mutations D87N, D87Y, S83F, *parE* H462Y and *qnrB2* were detected in one domestic and one travel-associated isolate each, *gyrA* mutation in 1-2 isolates each.

Among the five genes conferring resistance to gentamicin, *acc(3)*-IId was detected in four domestic and four travel-associated isolates, *aac(3)-IIe* in two domestic and two travel-associated isolates, *aac(3)-IVa* in three domestic and in one travel-associated isolate, *aac(3)-IIg* and *aac(6)-IIc* each in one domestic isolate.

Four unique genes conferring resistance to third-generation cephalosporins were detected, *blaCTX-M-55* was detected in four travel-associated isolates, *blaCTX-M-14* in one domestic and two travel-associated isolates, *blaCTX-M-1* and *blaSHV-12* each in two domestic isolates.

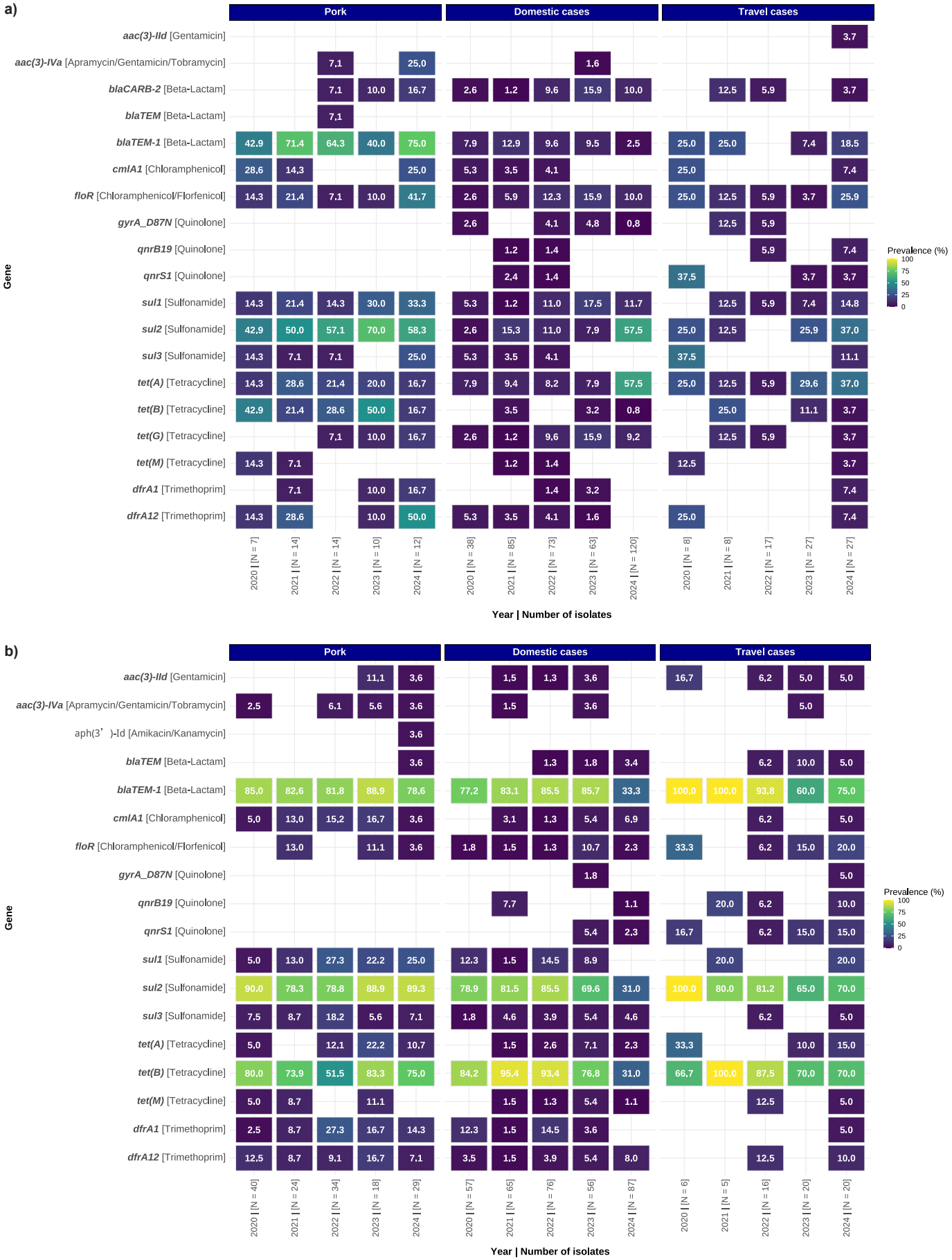
Two domestic and two travel-associated isolates carried *mph(A)* gene conferring resistance to azithromycin.

In 2022 and in 2024, single isolates carried colistin resistance genes *mcr-1* and *mcr-3*. Determinants conferring resistance to carbapenems were not detected.

In summary, the occurrence of AMR determinants differed between *S.* Typhimurium and monophasic *S.* Typhimurium isolates from humans. The differences in the most frequently detected genes were largely driven by the abundance of certain clones. Quinolone resistance determinants showed greater diversity in *S.* Typhimurium isolates - particularly those from travel-associated cases-than in monophasic *S.* Typhimurium. Moreover, the occurrence of certain quinolone resistance determinants differed between the two serotypes.

Figure 6.4 Presence (%) of genetic determinants of antimicrobial resistance among *S.* Typhimurium (a) and its monophasic variant (b) from domestic pork and human cases, Denmark, 2020-2024

DANMAP 2024



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

Resistance determinants in pork monophasic *S.* Typhimurium isolates

From 2020 to 2024, monophasic *S.* Typhimurium isolates from Danish pork showed markedly high presence of resistance genes conferring resistance to beta-lactams (penicillins; *blaTEM-1*), sulfamethoxazole, predominantly carrying the *sul2* gene, and tetracycline, with the *tet(B)* gene found most (Figure 6.4B). This is a known characteristic of the most frequent monophasic *S.* Typhimurium clone (ST34) circulating within the EU. The ASuT MDR profile was identified in 105 monophasic *S.* Typhimurium isolates, showing equal results from phenotypic and WGS analysis. The most commonly found gene combination was *aph(3'')/lb-aph(6)-Id/aph(3')-Ia/**blaTEM-1**/**sul2**/**tet(B)***, present in 41 pork isolates.

Several genes were found that confer resistance to amikacin and gentamicin (aminoglycosides), such as the *aac(3)-IId, aac(3)-IVa I and aph(3')-Id* (Figure 6.4B). Resistance genes associated with macrolide resistance were rare. In 2024, one monophasic *S.* Typhimurium isolate from pork carried the *mph(A)* gene and another harbored the *mef(C)-mef(G)* tandem genes. Both resistant determinants are associated with azithromycin resistance.

In 2020 and 2023, no resistance genes conferring macrolide resistance were found. While in 2021 and 2022, two monophasic *S.* Typhimurium isolates carried the *acrB_R717Q/L* substitution.

Genes conferring resistance to high-priority or last-resort antimicrobials were not detected in monophasic *S.* Typhimurium from pork, consistent with phenotypical testing (Figure 6.4B and Table 6.3).

Conclusions and future perspectives

WGS-based analysis enabled the identification of AMR determinants not only for phenotypically tested antimicrobials but also for those not tested routinely. In addition, it allowed detection of co-occurring AMR determinants within the same isolate and their association with circulating *Salmonella* clones. Future efforts should focus on assessing concordance between phenotypic and genotypic testing to support the potential replacement of phenotypic testing with WGS.

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