7

RESISTANCE IN INDICATOR BACTERIA

7. Resistance in indicator bacteria

Highlights

Over the last 5-year monitoring period, there have been no statistically significant trends in the occurrence of **fully-susceptible indicator *E. coli*** from broilers, pigs or cattle. Notably, in 2024 compared to 2023, full-susceptibility occurrence increased by 11% in isolates from cattle and decreased by 8% in isolates from pigs.

The occurrence of **multidrug-resistant indicator *E. coli*** compared to the previous year remained relatively constant in broilers and pigs and visibly decreased in cattle, in a clear shift from the increasing trend observed in recent years. Combined resistance to ampicillin, sulfamethoxazole, and tetracycline (ASuT) continued to be the most common multidrug-resistance profile among *E. coli* from pigs, however the relative occurrence of other profiles increased in 2024.

Compared to 2023, the **occurrence of resistance** to most antimicrobials in the test panel suffered a decrease in isolates from broilers and cattle, and an increase in isolates from pigs. The fluctuations between the two years were minor among isolates from broilers and pigs (1-5%), and more pronounced for cattle isolates (5-12%). Among pig isolates (although by a limited magnitude), there was an increase in occurrence of resistance to aminoglycosides (amikacin and gentamicin) and macrolides (azithromycin).

As in previous years, no colistin, meropenem or tigecycline resistance were detected in indicator *E. coli*. Resistance to amikacin, gentamicin, third generation cephalosporins and azithromycin were either absent or detected at low levels. Resistance to ciprofloxacin continued to be low in isolates from cattle and pigs and continued decreasing in broiler isolates.

As in previous years, samples from broilers, broiler meat and turkey meat examined for **carbapenemase-producing (CP) *E. coli*** (including OXA-48) were found negative. The occurrence of **ESBL- or AmpC- producing *E. coli*** remained constant below 5% in samples from broilers and domestic broiler meat, while it increased in samples from imported broiler meat and decreased in samples from imported turkey meat, by approximately 20%, compared to 2022.

7.1 Introduction

Escherichia coli and *Enterococcus* are included in the DANMAP programme to monitor the occurrence of antimicrobial resistance (AMR) in different reservoirs throughout the food chain for the following reasons: i) they are present as commensals in the gut microbiota of healthy animals and humans, ii) they can acquire antimicrobial resistance both via mutations in chromosomal genes and horizontal transfer of antimicrobial resistance genes, and iii) they have the potential to cause infections in both animals and humans, and to transfer antimicrobial resistance to pathogenic bacteria of the same or other species.

E. coli exhibiting resistance to third generation cephalosporins via the production of extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases (AmpCs) is among the fastest spreading antimicrobial resistance mechanisms in both humans and food-producing animals worldwide.

Carbapenemase-producing *Enterobacteriaceae* (CPE) pose a great threat to human health, as carbapenems are last-line antimicrobial drugs for the treatment of infections caused by multidrug-resistant Gram-negative bacteria.

Isolation and antimicrobial susceptibility testing of indicator *E. coli*, indicator enterococci and extended-spectrum cephalosporinase (ESC)- and carbapenemase (CP)-producing *E. coli* are performed in accordance with the rules for the EU harmonised monitoring of antimicrobial resistance [Decision 2020/1729/EU].

In 2024, isolates were obtained from randomly selected caecal samples collected from healthy broilers, cattle (calves under one year of age), and fattening pigs at slaughter. Additionally, for the specific monitoring of ESC- and CP-producing *E. coli*, fresh meat from broilers and turkeys was collected at retail. Details on sampling, analysis, susceptibility testing and interpretation of results are presented in Chapter 10.

7.2 Indicator *Escherichia coli*

Indicator *E. coli* isolates were obtained from 97%of caecal samples from broiler flocks 173/178, 90% of samples from pigs 184/205 and 93% of samples from cattle 154/166.

7.2.1 Indicator *Escherichia coli* from broilers, cattle and pigs

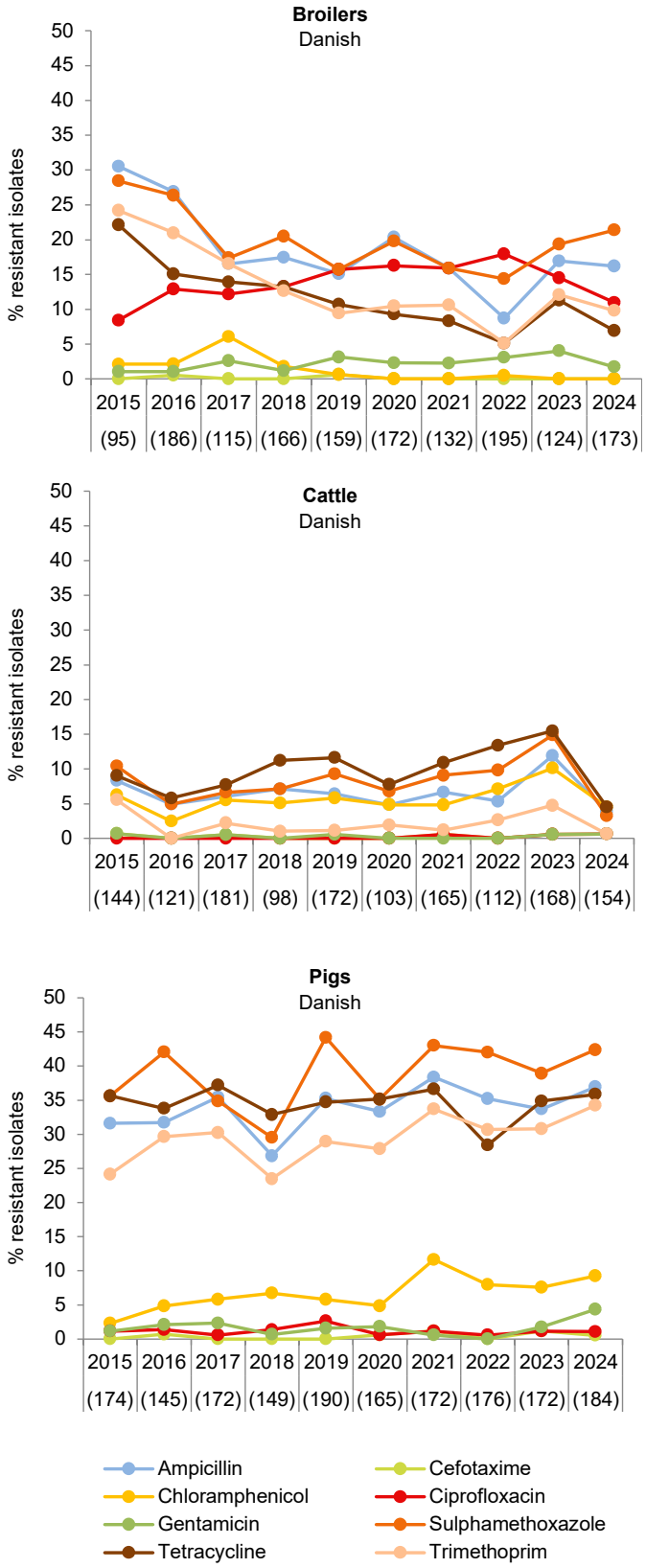
There has been no statistically significant increasing or decreasing trend in the annual prevalence of fully susceptible *E. coli* isolates from broilers, cattle or pigs during the past five years of monitoring (Figure 7.2) (p-values of 0.22 for broilers, 0.28 for pigs and 0.48 for cattle). Compared to 2023, the percentage of broiler isolates sensitive to all antimicrobials in the test panel increased by 4% in 2024, reaching the same value observed back in 2021 (64%). After the significant 5-year decrease in the proportion of fully susceptible *E. coli* in cattle, in 2024 it increased to 93% (similar to the level observed in 2020). Among isolates from pigs, the occurrence of full-susceptibility continued to decrease, and was at 40% in 2024, 8% lower than in 2023 (Table 7.1).

Table 7.1 Resistance (%) in *Escherichia coli* isolates from broilers, cattle and pigs, Denmark, 2024 DANMAP 2024

	Broilers	Cattle	Pigs
	Danish %	Danish %	Danish %
Amikacin	1	0	2
Ampicillin	16	4	37
Azithromycin	<1	<1	4
Cefotaxime	0	<1	<1
Ceftazidime	0	<1	<1
Chloramphenicol	0	5	9
Ciprofloxacin	11	<1	1
Colistin	0	0	0
Gentamicin	2	<1	4
Meropenem	0	0	0
Nalidixic acid	10	0	1
Sulphamethoxazole	21	3	42
Tetracycline	7	5	36
Tigecycline	0	0	0
Trimethoprim	10	<1	34
Fully susceptible (%)	64	93	40
Number of isolates	173	154	184

An isolate is considered fully susceptible if sensitive to all antimicrobial agents included in the test panel (Chapter 10, Table 10.3)

Figure 7.1 Resistance (%) among *Escherichia coli* isolates from broilers, cattle and pigs, Denmark, 2015-2024 DANMAP 2024



The number of isolates included each year is shown in the parentheses

Compared to 2023, the occurrence of resistance to most antimicrobials in the test panel showed an overall decrease in isolates from broilers and cattle, and an overall increase in isolates from pigs. The fluctuations between the two consecutive years were minor for broiler- and pig isolates (2-5% and 1-3%, respectively), and more pronounced for cattle isolates (5-12%) (Figure 7.1).

Remarkably, in *E. coli* from cattle, after an ongoing increase since 2021 in the occurrence of resistance to several tested antimicrobials, in 2024 the resistance levels markedly decreased, specifically for ampicillin (from 12% to 4%), chloramphenicol (from 10% to 5%), sulfamethoxazole (from 15% to 3%), tetracycline (from 15% to 5%) and trimethoprim (from 5% to <1%). While a shift in trend of tetracycline resistance could be explained by an ongoing decrease in consumption of tetracyclines in cattle, on the other hand, the consumption of amphenicols in calves continued to increase in 2024 (Chapter 4, Figure 4.5).

Also, the occurrence of resistance to ciprofloxacin in *E. coli* from broilers continued decreasing in 2024, after the decrease also observed in 2023, with fluoroquinolone resistance reaching levels similar to those of 2017 (11%). The results of 2023 and 2024 could represent a shift in the significant increasing trend observed in the decade leading to 2022.

Notably, in pig isolates (although by a limited magnitude), an increase in occurrence of resistance was observed from 2023 to 2024 for aminoglycosides (amikacin:<1% to 2%; gentamicin: 2% to 4%) and macrolides (azithromycin: 3% to 4%). These findings agree with the increases in antimicrobial consumption observed in recent years for aminoglycosides in weaners and macrolides in finishers (Chapter 4, Figure 4.4).

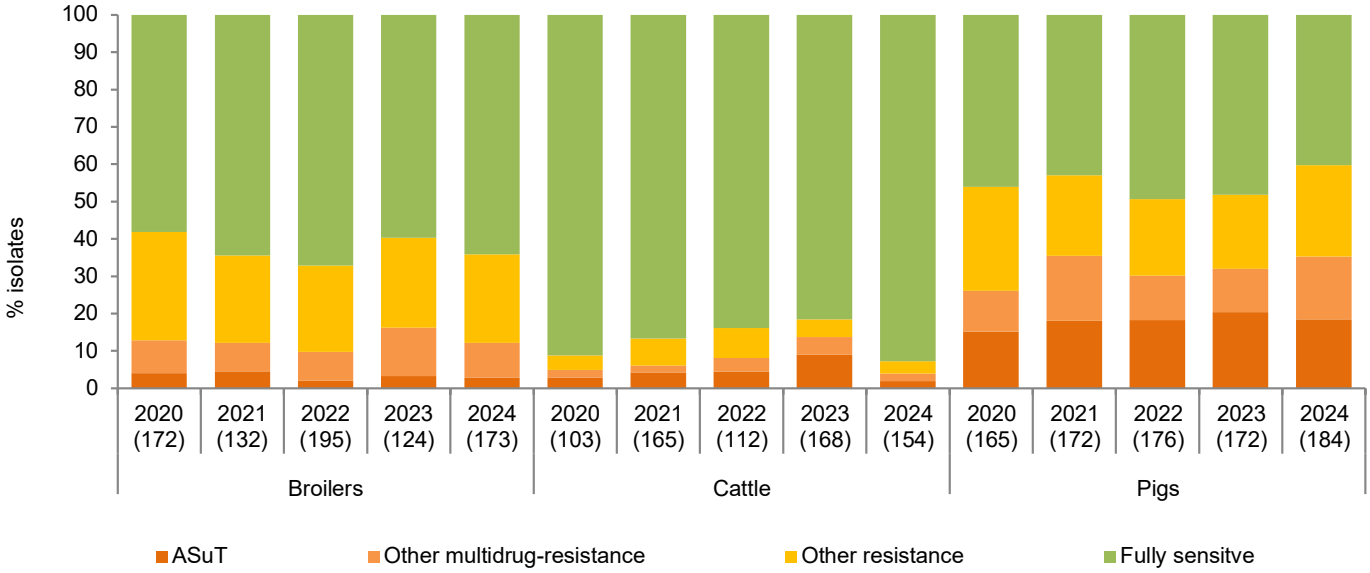
As in previous years, no isolates resistant to colistin, meropenem or tigecycline were detected. In 2024, amikacin resistance was detected in two isolates from broilers and three isolates from pigs. Azithromycin resistance was detected in seven isolates from pigs, and in single isolates from broilers and cattle. Resistance to third generation cephalosporins was not detected (for broilers) or detected at very low levels (up to 1%; for cattle and pigs) in indicator *E. coli* using non-selective methods. Resistance to fluoroquinolones continues to be very low (up to 1%) in isolates from cattle and pigs, and higher (11%) among broiler isolates (Table 7.1).

In 2024, the occurrence of multidrug-resistance (MDR) in *E. coli* from broilers, including combined resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT) and other MDR profiles, returned to an overall level (12%) similar to that prior to 2023. In pigs, occurrence of MDR and other resistance profiles continues to appear relatively stable, although with a 4% increase in the percentage of isolates resistant to less than three antimicrobial classes, and a 5% increase in MDR profiles other than ASuT in 2024 compared to 2023.

Hence, ASuT resistance continued to be the predominant MDR profile in isolates from pigs (18%), although in 2024 its occurrence was only higher than the occurrence of other MDR profiles by 1%. Notably, in cattle, there was a clear shift in the ongoing increasing trend in the occurrence of MDR *E. coli*, and in 2024 the overall MDR level decreased to 4% (2% ASuT and 2% other MDR profiles) (Figure 4.2).

Among indicator *E. coli* isolated with a non-selective procedure, presumptive ESBL/AmpC-producing isolates were found in single samples from pigs and cattle (Table 7.1) After testing with the second antibiotic panel for confirmation of ESBL/AmpC-producing phenotype, both isolates showed resistance to third- and fourth-generation cephalosporins (cefotaxime, ceftazidime and cefepime).

Figure 7.2 Relative distributions (%) of fully susceptible, resistant and multidrug-resistant *Escherichia coli* isolates from broilers, cattle and pigs, Denmark, 2020-2024
DANMAP 2024



The number of isolates included each year is shown in parentheses. An isolate is considered fully susceptible if sensitive to all antimicrobial agents included in the test panel, and multidrug-resistant if resistant to 3 or more of the 12 antimicrobial classes included in the test panel (Chapter 10, Table 10.3). ASuT are the multidrug-resistant isolates resistant to ampicillin, sulphamethoxazole and tetracycline

7.3 ESBL/AmpC- and carbapenemase-producing *E. coli*

In 2024, caecal samples collected from broilers at slaughter, and from packages of fresh, chilled broiler- and turkey meat collected from Danish wholesale and retail outlets were subject to the specific monitoring of ESBL/AmpC- and carbapenemase (CP)-producing *E. coli*.

Among the samples randomly collected at retail, 14% (41 out of 301) of broiler meat and 100% of turkey meat were imported products. The distribution of the country of origin of the sampled imported turkey meat did not change considerably compared to 2022, with a single country representing 93% of all samples in 2024 (previously 98%). Contrarily, the distribution of the country of origin of the sampled imported broiler meat changed compared to the previous reporting year. In 2022, the collected samples originated from three producing countries, with a predominating country (A) representing 80% of the sampled imported broiler meat. In 2024, the distribution shifted, and a different predominating country (B) of origin was observed representing 56% of all samples collected (an increase of 38% compared to 2022).

As in previous years, the selective procedures for detection of CP-producing *E. coli* (including oxacillinase-producing OXA-48-like enzymes), recovered no isolates.

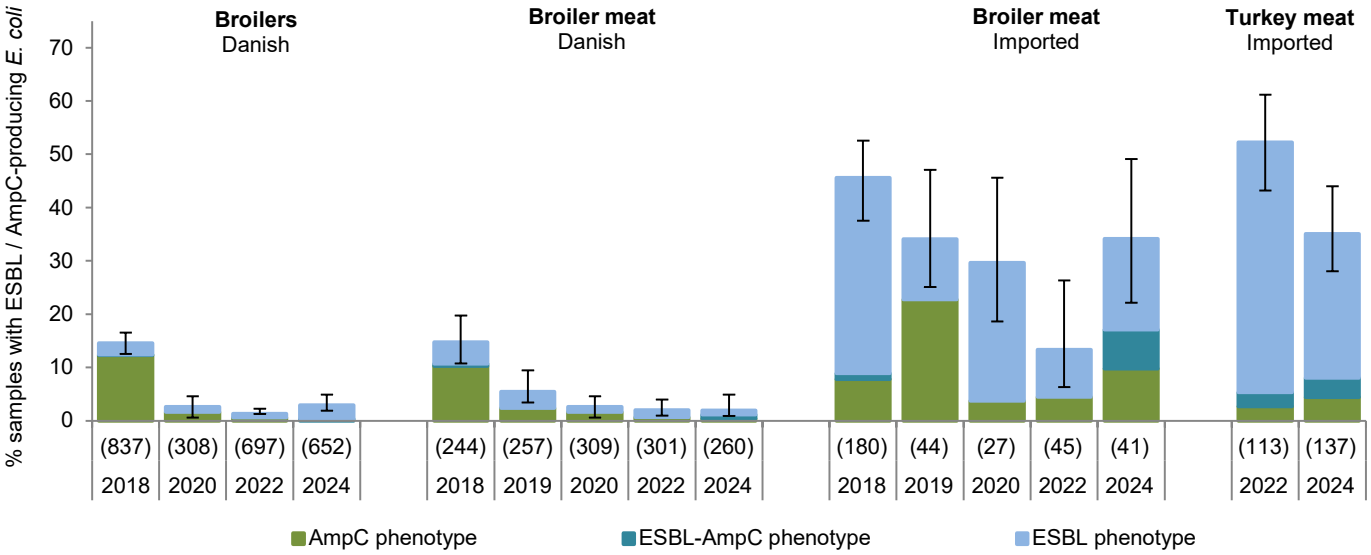
7.3.1 Prevalence of ESBL/AmpC-producing *E. coli* in broilers, and broiler- and turkey- meat

Following selective enrichment, ESBL/AmpC-producing isolates, i.e. *E. coli* resistant to third generation cephalosporins (cefotaxime and/or ceftazidime), here abbreviated as ESBL/AmpC *E. coli*, were obtained from 19/652 samples from broilers (3%; CI 95%: 2-5%), 5/260 samples from Danish broiler meat (2%; CI 95%: 1-5%), 14/41 samples from imported broiler meat (34%; CI 95%: 22-49%), and 48/137 samples from imported turkey meat (35%; CI 95%: 27-43%) (Table 7.2, Figure 7.3).

In 2024, in comparison to 2022, the prevalence of ESBL/AmpC *E. coli* has increased marginally in Danish broilers (from 1% to 3%) and remained the same in domestic broiler meat (2%). Notably, it increased markedly in imported broiler meat (from 13% to 34%) and decreased in the same magnitude in imported turkey meat (from 52% to 35%) (Figure 7.3, Table 7.2). ESBL/AmpC *E. coli* continued to show higher occurrence in imported broiler meat compared to Danish broiler meat.

Figure 7.3 Occurrence (%) of samples with phenotypic ESBL- or AmpC-producing *E. coli* from animals and meat recovered by selective enrichment, Denmark 2018-2024

DANMAP 2024



Number of samples tested per year is presented in the parentheses. Confidence intervals for total proportion of samples positive for phenotypic ESBL and/or AmpC producing *E. coli* - calculated as 95% binomial proportion Wilson intervals. Classification of ESBL and AmpC phenotypes according to the scheme provided by EFSA (Chapter 10, Section 10.7.2)

After a sharp decrease from 2018 to 2020 on the occurrence of ESBL/AmpC *E. coli* in domestic broilers and broiler meat, the levels seemed to have stabilized below 5% since then. On the contrary, in imported broiler meat, after the decrease observed in the previous four monitoring years, the occurrence of ESBL/AmpC *E. coli* increased markedly in 2024, returning to the level observed in 2019. This increase in ESBL/AmpC *E. coli* prevalence can likely be explained by the shift in the origin of the imported broiler products observed in 2024. In previous years, the sampled imported broiler meat originated predominantly from a country (A) with low occurrence of ESBL/ AmpC *E. coli* in broiler products. In 2024, most sampled imported broiler meat originated from a country (B) with a considerably higher prevalence of ESBL/ AmpC *E. coli* in broiler products. The presence of ESBL/AmpC Ec in imported turkey meat in Denmark has been monitored in 2022 and 2024, according to EU Decision 2020/1729. While the level was manifestly higher in imported turkey compared to imported broiler in 2022, in 2024 a similar prevalence was observed in both meat categories (Figure 7.3).

7.3.2 Phenotypic resistance in ESBL/AmpC-producing *E. coli*

In 2024, the relative frequency of ESBL-producing and/or AmpC-producing phenotypes remained mostly unchanged in comparison to previous years, except among isolates from imported broiler meat, which showed an increase in the relative occurrence of the AmpC-producing phenotype (10% of samples; 29% of ESBL/AmpC *E. coli* isolates) and of the combined ESBL-AmpC-producing phenotype (7% of samples; 21% of ESBL/AmpC *E. coli* isolates). In isolates recovered from imported turkey meat, the ESBL-producing phenotype continued to be predominant (27% of samples; 79% of ESBL/AmpC *E. coli* isolates) (Figure 7.3, Table 7.2).

As in previous years, all the recovered ESBL/AmpC *E. coli* isolates from broilers and Danish broiler meat were resistant to both third generation cephalosporins (cefotaxime and ceftazidime) and ampicillin. In 2024, those isolates were also all resistant to fourth generation cephalosporins (cefepime). Resistance to cefotaxime, ceftazidime and ampicillin was also very high (93% to 100%) in isolates from imported broiler- and turkey meat. Unlike previous years, cefepime resistance was found at a higher prevalence among the isolates recovered from domestic broiler meat (100%) than among those from imported broiler meat (71%) and imported turkey meat (90%) (Table 7.2).

A 17% increase in the occurrence of cefepime resistance among isolates from domestic broilers and broiler meat was already observed in 2022. Again in 2024, an increase of 33% was observed compared to the previous monitoring year. On the contrary, the occurrence of cefepime resistance has decreased in 2022 (by 17%) and in 2024 (by 12%) among ESBL/AmpC *E. coli* from imported broiler meat. Note that these results are based on a very low number of isolates recovered from domestic meat (six in 2022 and five in 2024).

In the same two monitoring years, the observed resistance to fluoroquinolones (ciprofloxacin) has markedly decreased in ESBL/AmpC *E. coli* from Danish broilers (from 44% in 2022 to 0% in 2024) and remained at a similar level in Danish broiler meat (17% in 2022 and 20% in 2024). In contrast to what was observed in 2022, in 2024 ciprofloxacin resistance also decreased in isolates from imported broiler meat (from 100% to 86%), as well as in isolates from imported turkey meat (from 83% to 69%).

Unlike the previous monitoring year, in 2024 ertapenem resistance was not observed in isolates from broilers. Among isolates from imported turkey meat, two out of 48 isolates (4%) were ertapenem-resistant, and as in 2022, no resistance to meropenem or imipenem was observed. ESBL-producing *E. coli* may present ertapanem-resistant, meropenem-sensitive phenotypes [Black, et al. 2024. *Antibiotics* 13(2), 185]. Such isolates rarely encode carbapenemase genes and show distinct clinical and microbiological characteristics compared to CP-producing *E. coli* [Adelman, et al. 2021. *Open Forum Infectious Diseases*, 9(1)].

The occurrence of colistin-resistant ESBL/AmpC *E. coli* among imported turkey meat decreased from 7% in 2022 to 4% in 2024.

Azithromycin resistance was observed in two out of 14 isolates from imported broiler meat (14%, 3% lower than in 2022) and in a single isolate from imported turkey meat (2%, as in 2022). Notably, the occurrence of gentamicin resistance decreased from 10% in 2022 to 4% in ESBL/AmpC *E. coli* from imported turkey meat, but it increased from 0% to 29% in isolates from imported broiler meat.

As in 2022, resistance to tigecycline, temocillin, meropenem and imipenem was not observed among the isolates collected in 2024, and no resistance to colistin was observed in ESBL/AmpC *E. coli* from broilers and broiler meat (Table 7.2).

Table 7.2 Resistance (%) and ESC resistance phenotypes in specific monitoring of ESC-producing *Escherichia coli* from animals and meat recovered by selective enrichment, Denmark, 2024 DANMAP 2024

	Broilers	Broiler meat		Turkey meat
Antimicrobial agent	Danish %	Danish %	Import %	Import %
Amikacin	0	0	0	0
Ampicillin	100	100	100	100
Azithromycin	0	0	14	2
Cefepime	100	100	71	90
Cefotaxime	100	100	100	100
Cefotaxime/clavulansyre	0	20	29	13
Cefoxitin	11	60	50	23
Ceftazidime	100	100	93	100
Ceftazidime/clavulansyre	0	20	29	13
Chloramphenicol	0	20	64	60
Ciprofloxacin	0	20	86	69
Colistin	0	0	0	4
Ertapenem	0	0	0	4
Gentamicin	0	0	29	4
Imipenem	0	0	0	0
Meropenem	0	0	0	0
Nalidixic acid	0	20	86	58
Sulfonamide	0	0	57	73
Temocillin	0	0	0	0
Tetracycline	16	20	57	81
Tigecycline	0	0	0	0
Trimethoprim	0	0	57	40
Number of AmpC phenotypes	0	1	4	6
Number of ESBL phenotypes	17	2	7	37
Number of ESBL+AmpC phenotypes	2	2	3	5
Number of ESC isolates (%)	19 (3%)	5 (2%)	14 (34%)	48 (35%)
Number of samples	652	260	41	137

Classification of ESBL-, AmpC- and AmpC+ESBL phenotypes is based on the MIC results (Chapter 10, Section 10.7.2). AmpC, ESBL and AmpC+ESBL phenotypes indicate the number of isolates expressing each specific phenotype

7.3.3 Genotypic resistance in ESBL/AmpC-producing *E. coli*

The genetic basis for ESBL and AmpC enzymes was detected in all isolates recovered by selective enrichment. The detected enzymes corresponded to the phenotypes derived from the susceptibility testing for most isolates, except 12 (two from broilers, two from domestic broiler meat, three from imported broiler meat and five from imported turkey meat). In those isolates, susceptibility testing revealed an ESBL- and AmpC-producing phenotype, while whole genome sequencing only revealed the presence of genes encoding for ESBL enzymes. The ESBL genotypes in those isolates were mostly due to single ESBL-encoding genes (CTX-M-1, CTX-M-15, CTX-M-27, CTX-M-55 and TEM-52B). However, two isolates from imported turkey meat showed the presence of more than one gene, including less frequent variants and also genes with unknown ESBL predicted phenotypes (Tables 7.2 and 7.3).

Among the AmpC-producing isolates recovered in 2024, resistance was, as observed in previous years, conferred by upregulated AmpC promotor C-42T mutations (seven isolates), followed by the CMY-2 plasmid-mediated AmpC enzyme (four isolates) (Table 7.3).

Among all ESBL-producing isolates, 10 different ESBL-encoding genes were detected. Overall, the most commonly observed gene across all categories of animals and meat sampled in 2024 was CTX-M-1, as observed in the previous reporting year. CTX-M-55 and CTX-M-15 were the following most abundant genes. As observed in 2022, among isolates from imported turkey meat, the encoding gene CTX-M-27 was considerably frequent, all ten different detected enzymes were observed, and seven isolates (15% of all ESBL Ec from imported turkey meat) had more than one ESBL-encoding gene (Table 7.3).

In total, 36 MLSTs were observed among all ESBL/AmpC-producing *E. coli* isolates. The most common MLSTs were ST10 and ST297 in Danish broilers, ST69 and ST162 in imported broiler meat, and ST10 and ST569 in imported turkey meat.

Table 7.3 Number of ESBL and AmpC enzymes detected in beta-lactamase-producing *E. coli* isolates from animals and meat recovered by selective enrichment, Denmark, 2024 DANMAP 2024

	Broilers	Broiler meat		Turkey meat
Enzymes	Danish	Danish	Import	Import
CTX-M-1	9	2	2	11
CTX-M-15			4	6
CTX-M-27				8
CTX-M-32				1
CTX-M-55			3	10
SHV-12			1	4
TEM-106				5
TEM-126				5
TEM-207				2
TEM-52B	10	2		2
CMY-2		1	2	1
Chromossomal AmpC (C-42T)			2	5
Number of AmpC genotypes		1	4	6
Number of ESBL genotypes (two or more enzymes)	19	4	10	42 (7)
Number (%) positive samples	19 (3%)	5 (2%)	14 (34%)	48 (35%)
Number of tested samples	652	260	41	137

Number (%) positive samples are isolates recovered by selective enrichment methods for monitoring of beta-lactamase-producing *E. coli* ESBL/AmpC enzymes were determined by whole genome sequencing of the recovered isolates (Chapter 10, Section 10.6)

7.4 Indicator *Enterococci*

Enterococci were obtained from 296 (98%) out of 303 faecal samples taken from broilers at slaughter, and antimicrobial susceptibility testing was subsequently performed on all 17 *E. faecalis* isolates and on 278 *E. faecium* isolates.

7.4.1 *E. faecalis* and *E. faecium* from broilers

Overall, 29% of the *E. faecalis* isolates and 85% of the *E. faecium* isolates were susceptible to all antimicrobials in the test panel. While the level decreased by 10% compared to 2022 for *E. faecalis*, it increased by 33% for *E. faecium* in the same period (Table 7.4).

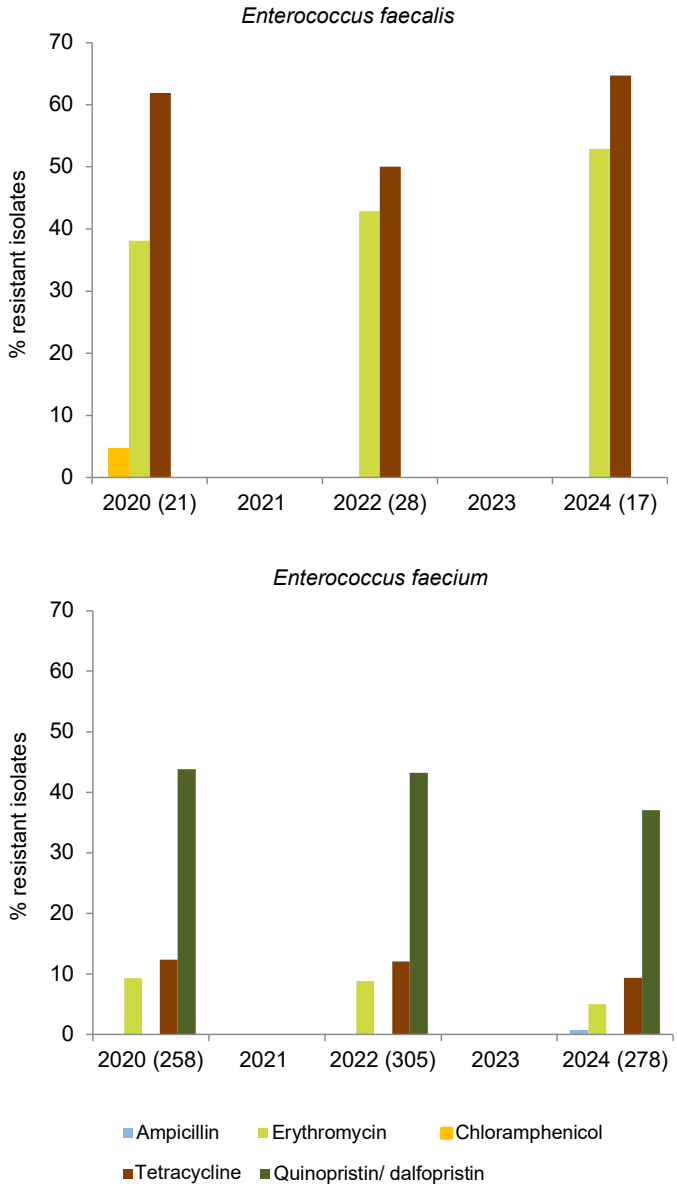
As in 2022, the previous year of monitoring in broilers, no enterococci isolates showed resistance to chloramphenicol, daptomycin, linezolid, gentamicin, teicoplanin, tigecycline or vancomycin. In 2024, ampicillin resistance was not observed in *E. faecalis*, but occurred in 1% of the *E. faecium* isolates. Resistance to erythromycin and tetracycline continued to be the most common. Compared to 2022, in 2024 resistance to these antibiotics showed an increase among *E. faecalis* (10% and 15%) and a decrease among *E. faecium* (4% and 3%). The increase in erythromycin resistance observed among *E. faecalis* isolates, follows a previous increase already observed in 2022 compared to 2020 (from 38% to 43%). The occurrence of ciprofloxacin resistance in *E. faecium* isolates was at the same level as in previous years (3%) (Table 7.4 and Figure 7.4).

Table 7.4 Resistance (%) in Enterococci isolates from broilers, Denmark, 2024 DANMAP 2024

Antimicrobial agent	*Enterococcus faecalis* %	*Enterococcus faecium* %
Ampicillin	0	1
Chloramphenicol	0	0
Ciprofloxacin	0	3
Daptomycin	0	0
Erythromycin	53	5
Gentamicin	0	0
Linezolid	0	0
Quinopristin/dalfopristin	-	37
Teicoplanin	0	0
Tetracycline	65	9
Tigecycline	0	0
Vancomycin	0	0
Fully susceptible (%)	29	85
Number of isolates	17	278

E. faecalis are assumed inherently resistant to streptogramins (Quinopristin/Dalfopristin)
An isolate is considered fully susceptible if sensitive to all antimicrobial agents included in the test panel (Chapter 10, Table 10.3)

Figure 7.4 Resistance (%) among Enterococci isolates from broilers, Denmark, 2024 DANMAP 2024



Number of isolates included each year is presented in the parentheses

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