7

RESISTANCE IN INDICATOR BACTERIA

7. Resistance in indicator bacteria

Highlights: Over the last five-year monitoring period, there have been no statistically significant trends in the prevalence of fully sensitive **indicator *E. coli*** from broilers or pigs. However, in the same period, there was a significant decrease in fully sensitive *E. coli* from cattle.

As in previous years, no colistin, meropenem or tigecycline resistance were detected in indicator *E. coli*. Amikacin resistance was detected in single isolates from broilers and pigs. Resistance to ciprofloxacin continued to be low in cattle and pigs, and after the increase observed in the previous year, it has decreased by 3% in broilers. Similarly to 2022, in 2023, azithromycin resistance was detected in a small number of isolates from pigs (3%) and additionally in 2% of broiler isolates. Additionally, in cattle, increases between 2% and 7% were observed in the resistance to ampicillin, chloramphenicol, sulfamethoxazole, tetracycline and trimethoprim.

The relative occurrence of multidrug-resistant indicator *E. coli* compared to the previous year increased in broilers and cattle, however a significant increasing trend over the past five years was only detected for cattle. Combined resistance to ampicillin, sulfamethoxazole, and tetracycline (ASuT) continued to be the most common multidrug-resistance profile among *E. coli* from cattle and pigs, however the relative occurrence of other profiles has increased in isolates from cattle in the past five years.

Importantly, as in previous years, samples from pigs, cattle and their meat examined for **carbapenemase-producing (CP) *E. coli*** (including OXA-48) were found negative.

The occurrence of **beta-lactamase-producing *E. coli***, obtained through selective procedures, continued the decreasing trend observed since 2019 in Danish cattle and pigs, and in imported pork, and was also lower in beef (domestic and imported) in 2023, compared to 2021. Antimicrobial susceptibility testing of ESBL-/AmpC-producing *E. coli* from imported beef showed, like in previous years, a very high (100%) occurrence in resistance to fourth generation cephalosporins (cefepime). Isolates from pigs and pork (domestic and imported) showed a decrease in occurrence of resistance to cefepime, compared to 2021. A single isolate from imported pork was found resistant to ertapenem, while one isolate from cattle and three isolates from pigs were found resistant to imipenem.

Whole genome sequencing of beta-lactamase-producing *E. coli* revealed ESBL, AmpC and ESBL+AmpC genotypes. All but one AmpC genotypes encoded upregulated AmpC promotor C-42T mutations, which was also observed in two ESBL+AmpC genotypes, one from pigs and another from cattle. Among the ESBL genotypes, 11 different ESBL genes were detected, with the most frequent being *CTX-M-1* and *CTX-M-15*.

In 2023, 22% of ***E. faecalis*** isolated from pigs were fully sensitive. None of the isolates showed resistance to ampicillin, ciprofloxacin, linezolid, teicoplanin, tigecycline or vancomycin. Combined resistance to chloramphenicol, tetracycline and erythromycin was the most common resistance profile. The overall decrease in resistance observed in 2021 did not continue in 2023.

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 3rd 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. Some studies have suggested a zoonotic transmission of ESBL/AmpC-producing *E. coli* [Liu et al 2023. One Health, 16: 100518; Roer et al 2019. J Antimicrob Chemother, 74(3):557; Mughini-Gras et al 2019. Lancet Planet Health, 3(8): e357-e369; Liu et al 2018. mBio, 9(4): e00470-18], thus the occurrence of ESBL/AmpC- producing *E. coli* in food-producing animals and their meat is considered of public health relevance. Accordingly, in the harmonized EU monitoring of AMR in zoonotic and indicator bacteria, this is considered a key outcome indicator [EFSA/ECDC 2024, EFSA Journal 2024; 22(2):e8583], for which trends are assessed annually at EU- and Member State level. The zoonotic nature of ESBL/AmpC-producing *E. coli* isolated in Denmark from humans, animals and meat is addressed in Chapter 3.

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. The monitoring of CP-producing *E. coli* is mandatory in healthy food-producing animals (broilers, fattening turkeys, fattening pigs and calves) and their derived meat. In recent years, CP-producing *E. coli* have been increasingly detected in food-producing animals in the EU, which raises the concern that animals might become a CPE reservoir in the future [EFSA/ ECDC 2024, EFSA Journal 2024; 22(2):e8583].

Isolation and antimicrobial susceptibility testing of indicator *E. coli*, indicator enterococci and ESBL-/AmpC-producing and carbapenemase (CP)-producing *E. coli* are performed in accordance with the EU harmonised monitoring of antimicrobial resistance [Decision 2020/1729/EU]. In 2023, 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 ESBL-/AmpC- and CP-producing *E. coli*, fresh meat from pigs and cattle was collected at retail and at border control posts (BCPs). 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 (125/129), 98% of samples from pigs (173/176) and 98% of samples from cattle (169/172). These isolates were obtained with a non-selective isolation procedure. Results obtained by selective procedures for specific monitoring of ESBL-/AmpC- and CP-producing *E. coli* are presented in Section 7.3.

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

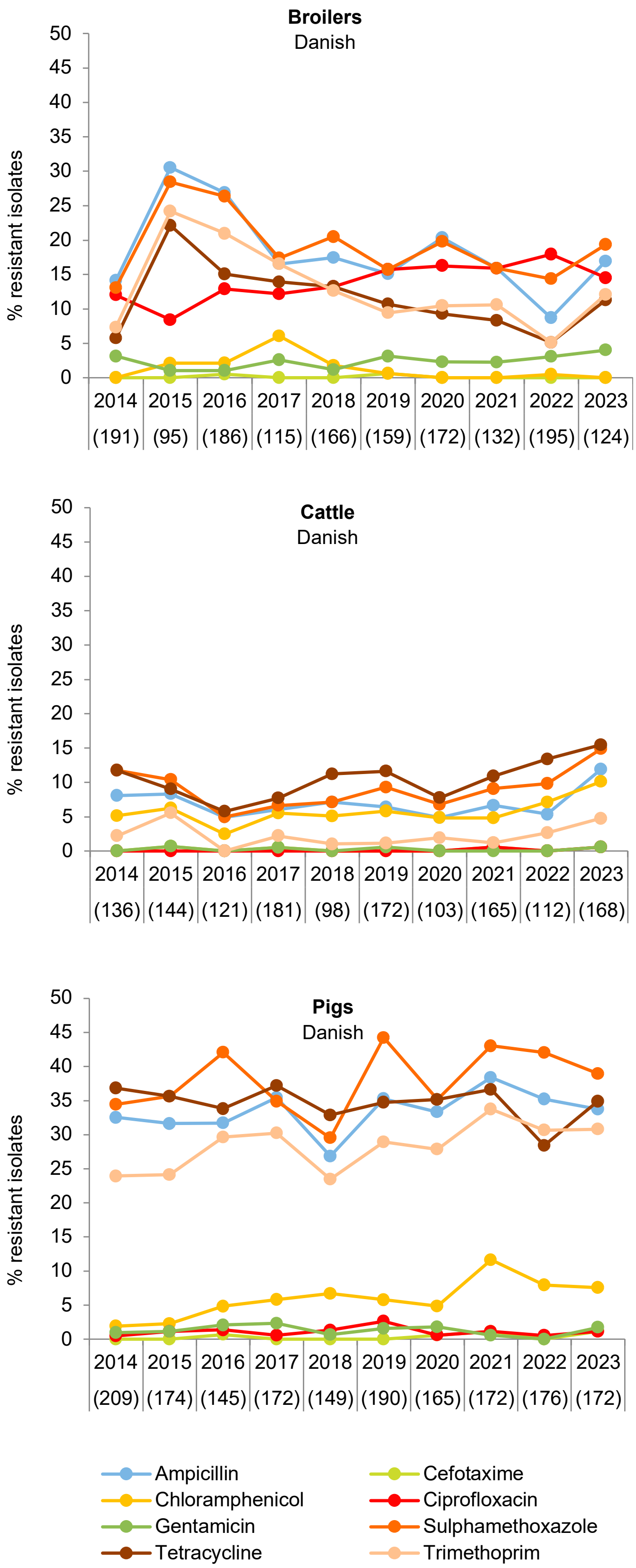
There has been no statistically significant increasing or decreasing trend in the annual prevalence of fully sensitive *E. coli* isolates from broilers or pigs during the past five years of monitoring (Figure 7.2) (p-values of 0.36 for broilers, and 0.10 for pigs). After a consecutive increase to 64% in 2021 and to 67% in 2022, in 2023 the percentage of broiler isolates susceptible to all antimicrobials in the test panel decreased to 60%, while compared to 2022, the percentage of fully sensitive pig isolates (48%) decreased by 1%. A significant decrease in the proportion of fully sensitive *E. coli* between 2019 and 2023 was detected in cattle (p-value=0.04). The percentage of fully sensitive cattle isolates (82%) continued in 2023 the decrease already observed in 2021 (to 87%) and 2022 (to 84%) (Table 7.1).

Table 7.1 Resistance (%) in *Escherichia coli* isolates from broilers, cattle and pigs, Denmark, 2023 DANMAP 2023

	Broilers	Cattle	Pigs
	Danish %	Danish %	Danish %
Amikacin	<1	0	<1
Ampicillin	17	12	34
Azithromycin	2	0	3
Cefotaxime	0	<1	1
Ceftazidime	0	<1	<1
Chloramphenicol	0	10	8
Ciprofloxacin	15	<1	1
Colistin	0	0	0
Gentamicin	4	<1	2
Meropenem	0	0	0
Nalidixic acid	15	0	0
Sulphamethoxazole	19	15	39
Tetracycline	11	15	35
Tigecycline	0	0	0
Trimethoprim	12	5	31
Fully sensitive (%)	60	82	48
Number of isolates	124	168	172

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

Figure 7.1 Resistance (%) among *Escherichia coli* isolates from broilers, cattle and pigs, Denmark, 2023 DANMAP 2023



The number of isolates included each year is shown in parentheses

Compared to 2022, the occurrence of resistance to most antimicrobials in the test panel suffered none or mostly minor fluctuations (1 to 3%). A few exceptions were found in isolates from broilers, where resistance to ampicillin, sulfamethoxazole, tetracycline and trimethoprim increased back to levels like those observed in 2021, after the decrease observed in 2022. The inverse was detected for resistance to fluoroquinolones, which decreased in 2023 back to levels similar to those of 2021. In isolates from pigs, resistance to sulfamethoxazole continued the decrease by 3% also observed in the previous year, while resistance to tetracycline increased by 11%, returning to a level similar to 2021. Although by a limited magnitude (between 2% and 5%), an increase in the percentage of resistance to chloramphenicol, sulfamethoxazole, tetracycline and trimethoprim was observed in *E. coli* from cattle, similarly to the trend seen in 2022. Additionally, the occurrence of resistance to ampicillin among cattle isolates increased by 7% from 2022 to 2023 (Figure 7.1).

As in previous years, no isolates resistant to colistin, meropenem or tigecycline were detected. In 2023, amikacin resistance was detected in a single isolate from cattle and in a single isolate from broilers. Azithromycin resistance was, similarly to previous years, detected in 3% of the isolates from pigs, and notably detected in 2% of the broiler isolates. Azithromycin resistance has been previously detected in broiler isolates in 2015 (1%) and 2020 (<1%). As in previous years, resistance to 3rd generation cephalosporins was not detected or detected at very low levels (up to 1%) in indicator *E. coli* using non-selective methods (Table 7.1).

Resistance to fluoroquinolones continues to be very low (up to 1%) or not observed in *E. coli* from cattle and pigs, and it has notably decreased by 3% in 2023 compared to 2022 among broiler isolates (Figure 7.1). The following monitoring years will disclose whether this represents a shift in the trend of fluoroquinolone resistance or a simple oscillation on the significant increasing trend of the past 10-years of monitoring (p-value=0.01).

The occurrence of resistant and multidrug-resistant (MDR) *E. coli* in broilers did not continue the decrease observed in the two previous years. Instead, MDR increased from 10% in 2022 to 16% in 2023, mostly due to an increase (from 8% to 13%) in occurrence of MDR patterns other than resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT). In pigs, occurrence of MDR and other resistance patterns continues to appear relatively stable, with a 2% increase in the percentage of ASuT isolates in 2023 compared to 2022. In cattle, there is a significant increasing trend in the occurrence of multidrug-resistant *E. coli* since 2019 (p-value=0.005), and in 2023 there was a particularly marked increase in the percentage of ASuT isolates (9%), compared to the previous four monitoring years (Figure 7.2). ASuT resistance continues to be the predominant MDR profile in isolates from pigs (20%), and isolates from cattle (9%).

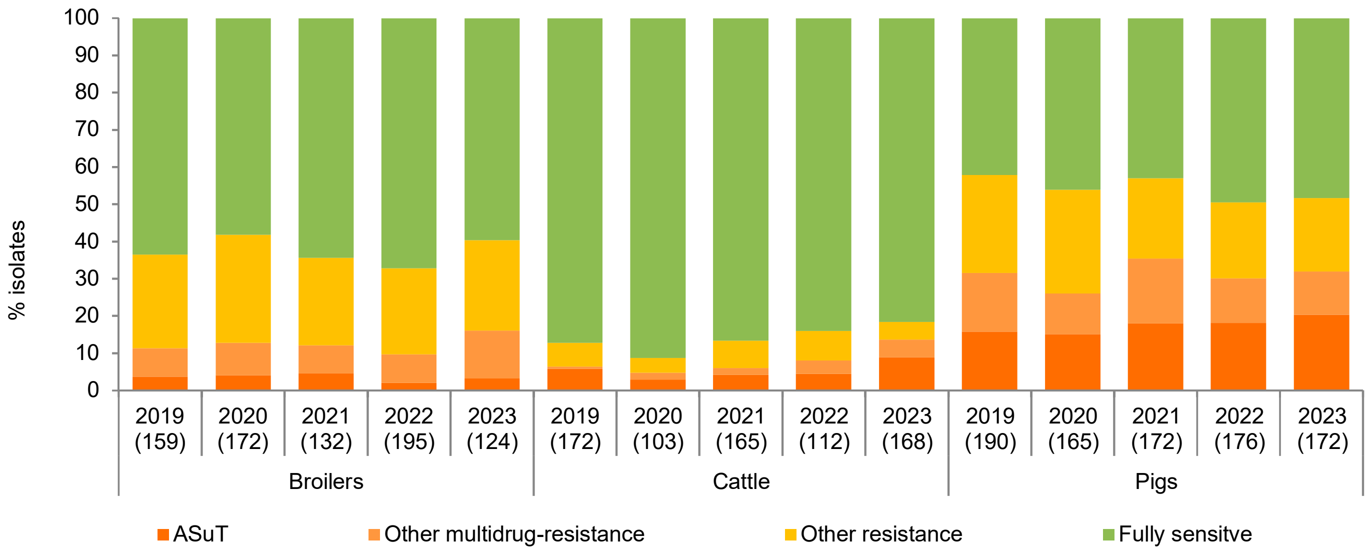
Among indicator *E. coli* isolated with a non-selective procedure, presumptive ESBL/AmpC-producing isolates were found in two samples from pigs and one sample from cattle (Table 7.1) After testing with the second antibiotic panel for confirmation of ESBL/AmpC-producing phenotype, one of the isolates from pigs did not show resistance to third- and fourth-generation cephalosporins.

7.2.2 Perspectives

At the EU level, full sensitivity in indicator *E. coli* isolated from broilers, pigs and cattle varies greatly between countries. The levels observed in 2023 in Denmark are all well above the observed EU median values in 2022 (19% in broilers, 38% in pigs and 54% in cattle). Overall, at EU level, and in many individual European countries, a significant increase in the occurrence of fully-sensitive isolates has been detected in broilers between 2014 and 2022. A similar trend has been observed in fewer individual countries, but not at the EU level, among isolates from pigs and cattle. No significant increasing or decreasing trends in full sensitivity were reported for Denmark between 2014 and 2022, individually, in the latest European Summary Report [EFSA and ECDC 2024, EFSA Journal 2024;22:e8583].

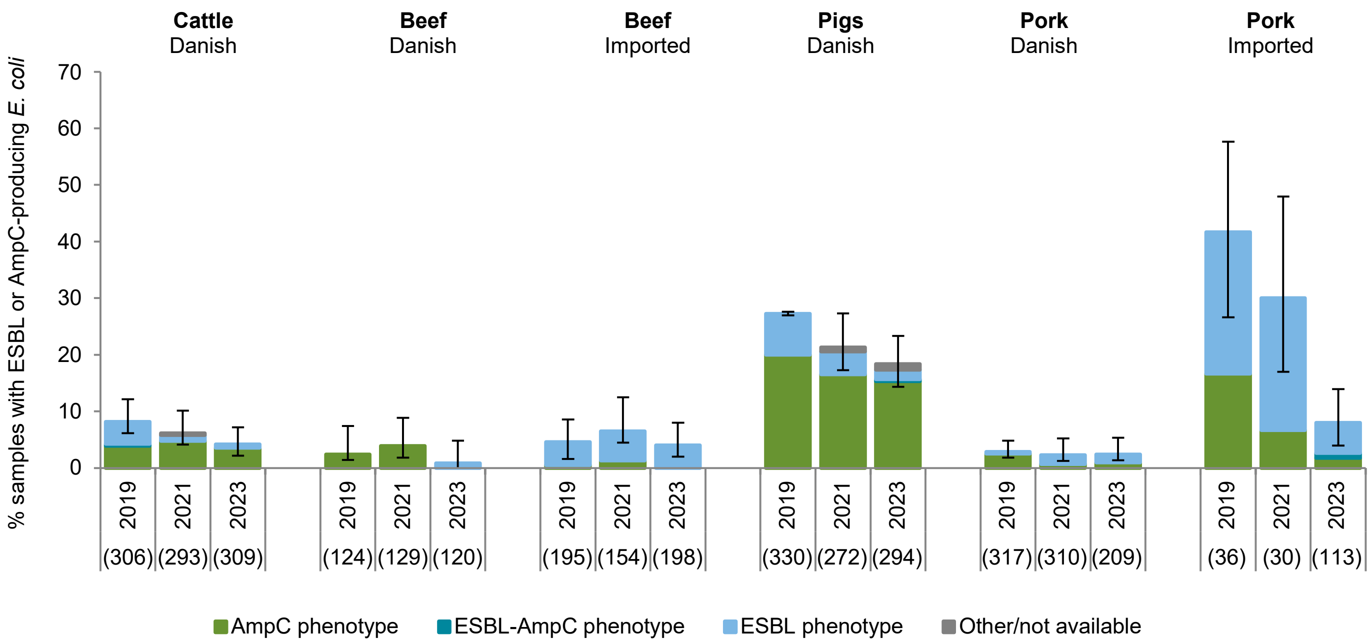
Accordingly, and as in previous years, in DANMAP 2023 no significant increasing or decreasing trends were observed in the occurrence of fully sensitive indicator *E. coli* recovered from broilers or pigs in the last five-year monitoring period (in DANMAP 2023, between 2019 and 2023). An increase of 36% in the systemic antibiotic treatment of young cattle has been observed since 2012. Also, the use of amphenicols and macrolides in calves, has been increasing steadily sinde 2014 and 2020, respectively (Chapter 4, Section 4.3.2). Such increases in consumption may explain the increasing trend in the occurrence of resistant and multidrug-resistant *E. coli* in calves. It is possible that different trends are detected when different monitoring periods are included in the analysis, and/or different methods are applied. In DANMAP 2022, no significant trend was observed among the same isolates in the period 2014 to 2022 [DANMAP 2022, Textbox 7.1].

Figure 7.2 Relative distributions (%) of fully sensitive, resistant and multidrug-resistant *Escherichia coli* isolates from broilers, cattle and pigs, Denmark, 2023
DANMAP 2023



The number of isolates included each year is shown in parentheses. 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 the 12 antimicrobial classes included in the test panel (Chapter 10, Table 10.3). ASuT are the multidrug-resistant isolates resistant to ampicillin, sulfamethoxazole and tetracycline

Figure 7.3 Occurrence (%) of samples with phenotypic ESBL- or AmpC-producing *E. coli* from animals and meat recovered by selective enrichment, Denmark, 2019-2023
DANMAP 2023



Number of samples tested per year is presented in parentheses. Classification of ESBL and AmpC phenotypes is based on the MIC results (Chapter 10, section 10.7.2). The total number of samples of imported beef include 192 samples collected at retail and 6 samples collected at border control posts (BCPs). The total number of samples of imported pork include 110 samples collected at retail and 3 samples collected at BCPs

At the EU level, a significant negative association has been again determined in the most recent JIACRA IV report between the probability of full sensitivity in indicator *E. coli* and the overall consumption of antimicrobials by food-producing animals, observed between 2018 and 2021 [ECDC, EFSA and EMA 2024, EFSA Journal 2024; 22:e8589]. The same report determined a significant decreasing trend in overall antimicrobial consumption by food-producing animals in 20 countries and a corresponding increasing trend in the proportion of fully sensitive indicator *E. coli* in 10 of those countries, including Denmark. JIACRA IV also showed a systematic trend analysis, with results indicated by combination of bacterial species and antimicrobial class. Here, in food-producing animals, statistically significant associations between decrease in antimicrobial use and decrease in antimicrobial resistance in indicator *E. coli* were detected for third- and fourth-generation cephalosporins (3 countries), fluoroquinolones (4 countries), polymyxins (3 countries), aminopenicillins (4 countries) and tetracyclines (14 countries). For Denmark, a significant association between use and resistance in indicator *E. coli* was only observed for polymyxins, specifically for the decrease in colistin use and resistance between 2014 and 2021. This was most likely due to the abrupt decrease to almost zero in the use of colistin in pigs that occurred in 2017 after the increase of the multiplication factor for colistin in the Yellow Card.

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

In 2023, caecal samples collected from pigs and cattle at slaughter, from packages of fresh, chilled pork and beef collected from Danish wholesale and retail outlets, and consignments of pork and beef meat sampled at Danish border control posts were monitored for the presence of extended-spectrum beta-lactamase (ESBL)- , cephalosporinase (AmpC)-, and carbapenemase(CP)-producing *E. coli*. In accordance with the harmonised EU monitoring rules [Decision 2020/1729/EU], packages of meat were collected at retail and border control posts without pre-selecting by country of origin. Of the samples randomly collected at retail, 34% of pig meat and 62% of cattle meat were imported products. Additionally, three samples of imported pork and six samples of imported beef were collected at border control posts.

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 ESBL-/AmpC-producing *E. coli* from pigs, pork, cattle and beef

Following selective enrichment, ESBL/AmpC-producing isolates, i.e. *E. coli* resistant to 3rd generation cephalosporins (cefotaxime and/or ceftazidime), were obtained from 54/294 samples from pigs (18%; CI 95%: 14-23%), 5/209 samples from Danish pork (2%; CI 95%: 1-5%), 9/113 samples from imported pork (8%; CI 95%: 4-14%) , 13/309 samples from cattle (4%; CI 95%: 2-7%), 1/120 samples from Danish beef (1%; CI 95%: 0-5%) and 8/198 samples from imported beef (4%; CI 95%: 2-8%) (Table 7.2, Figure 7.3). ESBL/AmpC-producing *E. coli* was not detected in samples collected at border control posts.

In 2023, in comparison to 2021, the prevalence of ESBL/AmpC-producing *E. coli* has overall decreased in all tested animal/food categories. Specifically, the proportion of cattle and beef samples that tested positive decreased by a magnitude of 2%, while prevalence among samples from Danish pigs decreased by 3% since 2021, and showed a significant decrease over the three last monitoring years (p-value = 0.02). Notably, the occurrence of ESBL/AmpC-producing *E. coli* decreased in imported pork from 42% in 2019 and 30% in 2021 to 8% in 2023. This large decrease needs to be interpreted with care due to the low number of samples tested in 2019 and 2021 (n=36 and n=30, respectively) in comparison to 2023 (n=113). In 2021, the 95% confidence interval for the proportion of positive imported pork samples was 17-48%, thus the occurrence of a decreasing trend may be a possibility, however it needs to be confirmed in the upcoming monitoring years. As observed previously, the occurrence of ESBL/AmpC-producing *E. coli* continues to be higher in imported meat compared to domestic meat (Figure 7.3). This difference is particularly obvious for pork, with a difference of 10% in ESBL/AmpC occurrence.

In 2023, the relative frequency of ESBL-producing and AmpC-producing phenotypes remained mostly unchanged in comparison to 2021, except among isolates from Danish beef, which showed a predominance of the ESBL-producing phenotype in 2023, unlike the two previous monitoring years (Figure 7.3).

All the recovered ESBL/AmpC-producing isolates were resistant to both 3rd generation cephalosporins (cefotaxime and ceftazidime) and to ampicillin. Isolates from imported beef were additionally 100% resistant to the 4th generation cephalosporin cefepime. In the previous monitoring year, imported beef isolates also presented the highest occurrence of cefepime resistance among all animal and meat categories sampled (90%). Compared to 2021, in 2023 the observed proportions of resistance to cefepime remained similar in isolates from cattle (with a 2% decrease) and decreased among isolates from pigs (by 19%), domestic pork (by 26%) and imported pork (by 11%) (Table 7.2).

In 2023, resistance to azithromycin was observed in one out of nine isolates (8%) from cattle, one out of eight isolates (13%) from imported beef, nine out of 54 (17%) isolates from pigs and in two out of five (40%) isolates from domestic pork, which represents an overall increase, compared to the proportions observed in 2021. The prevalence of resistance to ciprofloxacin was also higher in 2023 than in 2021 among isolates from cattle (three out of 13), imported beef (five out of eight), and imported pork (three out of nine).

As in 2021, no resistance to colistin or meropenem was observed in the specific monitoring of ESBL/AmpC-producing *E. coli*. However, ertapenem resistance was observed in one out of nine isolates (11%) from imported pork, while imipenem resistance was observed in one out of 13 isolates (8%) from cattle and in three out of 54 isolates (6%) from pigs.

As in previous years, resistance to tigecycline was not observed among the isolates collected in 2023, and temocillin resistance was detected in a single isolate from pigs. Resistance to sulfamethoxazole, tetracycline and trimethoprim remained common among the recovered ESBL/AmpC-producing isolates (Table 7.2).

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 the majority of the isolates. In one isolate from cattle, whole genome sequencing revealed a ESBL and AmpC-producing genotype, even though this was not detected with susceptibility testing, while another cattle isolate presented a AmpC phenotype and a ESBL genotype. One isolate from imported beef showed a ESBL phenotype, but a AmpC genotype. Among isolates from pigs, one isolate with AmpC phenotype did not show a AmpC genotypic profile, and three isolates without a ESBL or AmpC phenotype, were determined as ESBL-producers with whole genome sequencing. Finally, one isolate from imported pork showed a ESBL- and AmpC-producing phenotype, which was not confirmed in the genotype (Tables 7.2 and 7.3).

Among the AmpC-producing isolates recovered in 2023, resistance was, as observed in previous years, mainly conferred by upregulated AmpC promotor C-42T mutations (10 isolates from cattle, one from imported beef, forty-four from pigs, and two from Danish and imported pork). In a single isolate from pigs the mutation T-32A was observed instead.

Among all ESBL-producing isolates, 11 different ESBL genes were detected. Overall, the most commonly observed ESBL encoding genes across all categories of animals and meat sampled in 2023 were *blaCTX-M-1* and *blaCTX-M-15*, with the latter being most abundant among isolates from imported beef. The ESBL genes *blaCTX-M-32* and *blaSHV-12* were only detected among isolates from imported beef, while *blaOXA-10*, *blaTEM-15* and *blaTEM-52B* were only detected in isolates from cattle (Table 7.3).

Among the two isolates that harboured both ESBL and AmpC genotypes, upregulated AmpC promotor C-42T mutation was detected, together with the ESBL genes *blaOXA-1* in the pig isolate, and *blaOXA-10* in the cattle isolate (Table 7.3).

In total, 50 MLSTs were observed among all ESBL/AmpC- producing *E. coli* isolates. The most common MLST was ST88, followed by ST23.

7.3.2 Perspectives

There is an ongoing decreasing trend since 2019 in the occurrence of ESBL/AmpC-producing *E. coli* in cattle (although non-significant), pigs and imported pork (significant) (Figure 7.3). Similar decreasing trends have not been observed at EU-level between 2015 and 2021 [EFSA and ECDC 2024, EFSA Journal 2024;22:e8583].

Table 7.2 Resistance (%) and beta-lactam resistance phenotype distribution in ESBL/ApmC-producing *Escherichia coli* recovered from animals and meat by selective enrichment, Denmark, 2023 DANMAP 2023

	Cattle	Beef	Pigs	Pork	
Antimicrobial agent	Danish %	Import %	Danish %	Danish %	Import %
Amikacin	0	0	0	0	0
Ampicillin	100	100	100	100	100
Azithromycin	8	13	17	40	0
Cefepime	31	100	24	60	78
Cefotaxime	100	100	100	100	100
Cefotaxime/clavulanic acid	85	0	89	40	22
Cefoxitin	85	0	85	40	33
Ceftazidime	100	100	100	100	100
Ceftazidime/clavulanic acid	85	0	89	40	22
Chloramphenicol	15	38	19	0	0
Ciprofloxacin	23	63	6	0	33
Colistin	0	0	0	0	0
Ertapenem	0	0	0	0	11
Gentamicin	8	13	11	0	11
Imipenem	8	0	6	0	0
Meropenem	0	0	0	0	0
Nalidixic acid	8	25	4	0	33
Sulfamethoxazole	38	63	70	80	56
Temocillin	0	0	2	0	0
Tetracycline	54	50	57	80	67
Tigecycline	0	0	0	0	0
Trimethoprim	31	38	46	60	22
Number of AmpC phenotypes	11	0	45	2	2
Number of ESBL phenotypes	2	8	5	3	6
Number of ESBL+AmpC phenotypes	0	0	1	0	1
Other phenotypes	0	0	3	0	0
Number of isolates (%)	13 (4%)	8 (4%)	54(18%)	5 (2%)	9 (8%)
Number of samples	309	198	294	209	113

Classification of ESBL-, AmpC- and AmpC+ESBL-producing 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. Results for Danish beef are not shown since only a single ESBL-producing isolate was detected

At the EU-level, for pigs, the association between consumption of 3rd and 4th generation cephalosporins and cefotaxime resistance in indicator *E. coli* was significant both for 2019 and 2021. Similarly, despite the observed variations between countries in the consumption of 3rd and 4th generation cephalosporins and the reported occurrence of ESBL- and/or AmpC-producing *E. coli* under specific monitoring, significant positive associations were also observed between consumption and resistance, considering data from broilers, turkeys, pigs and cattle, collected in the period of 2018 to 2021 [ECDC, EFSA and EMA 2024, EFSA Journal 2024; 22:e8589].

In Denmark, 3rd and 4th generation cephalosporins are not used in the treatment of pigs or cattle. Only 1st generation cephalosporins have been used for intramammary treatments in cattle, and their use has been drastically decreasing since 2014, and was nearly absent in 2023 (Chapter 4, Table 4.1). Thus, a decrease in the consumption of 3rd and 4th generation cephalosporins is not likely to be the cause of the decrease in ESBL/AmpC-producing *E. coli* occurrence observed in Danish cattle and pigs, but it could justify the observed decreased in imported pork meat.

There is a large variation in the prevalence of ESBL-/AmpC-producing *E. coli* recovered from animals and meat in different EU countries. In 2021, prevalence ranged from 3% to 77% in pigs, 6% (in Denmark) to 59% in cattle, 0% to 19% in pork, and 0% to 31% in beef [EFSA and ECDC 2024, EFSA Journal 2024;22:e8583]. With the prevalence levels observed in 2023 (Table 7.2), Denmark continues to be among the countries with the lowest occurrence of beta-lactamase-producing *E. coli* in pigs, cattle and meat thereof.

The enzymes of the ESBL and AmpC-producing *E. coli* observed in 2023 are consistent with the enzymes detected in previous years, and also with the distribution of enzymes among isolates from pigs and cattle observed at EU-level [EFSA and ECDC 2024, EFSA Journal 2024;22:e8583].

The zoonotic transmission of beta-lactamase-producing *E. coli* continues to be investigated, with studies presenting different conclusions.

In the EU, a statistically significant association was found between resistance to 3rd generation cephalosporins in invasive *E. coli* from humans and in indicator *E. coli* from calves (2019) and broilers (2020), but not from pigs [ECDC, EFSA and EMA 2024, EFSA Journal 2024; 22:e8589]. In Chapter 3, ESBL/AmpC-producing *E. coli* isolates collected in Denmark between 2018 and 2023 from animals and meat and from human bloodstream infections were compared to address the likelihood of transmission between animals/meat and humans in Denmark.

Still, no carbapenemase-producing *E. coli* were detected in any of the samples tested in 2023. In the EU, in 2021, presumptive CP-producing *E. coli* was detected under specific phenotypic monitoring in two samples from fattening pigs. Additionally, CP-producing genotypes were detected with whole genome sequencing in 23 isolates from pigs and five isolates from cattle [EFSA and ECDC 2024, EFSA Journal 2024;22:e8583]. Since 2021 was the first year of mandatory specific monitoring of CP-producing *E. coli* in pigs and cattle (according to Decision 2020/1729/EU), it is possible that a higher number of isolates will be reported for 2023 at EU-level in the forthcoming EU Summary Report.

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, 2023 DANMAP 2023

	Cattle	Beef		Pigs	Pork	
Enzymes	Danish	Danish	Import	Danish	Danish	Import
blaCTX-M-1		1	1	5	3	2
blaCTX-M-15	1		3			1
blaCTX-M-27						1
blaCTX-M-32			2			
blaCTX-M-55				2		3
blaDHA-1	1			2		
blaOXA-1			1	1		
blaOXA-10	1					
blaSHV-12			1			
blaTEM-15	1					
blaTEM-52B	1					
Chromossomal AmpC (T-32A)				1		
Chromossomal AmpC (C-42T)	10		1	44	2	2
Number of AmpC genotypes	9	0	1	44	2	2
Number of ESBL genotypes	3	1	7	9	3	7
Number of AmpC+ESBL genotypes	1	0	0	1	0	0
Number (%) positive samples	13 (4%)	1 (1%)	8 (4%)	54 (18%)	5 (2%)	9 (8%)
Number of tested samples	309	120	198	294	209	113

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 169 (34%) out of 492 faecal samples taken from pigs at slaughter, and antimicrobial susceptibility testing was subsequently performed on 87 *E. faecalis* isolates. The identified *E. faecium* isolates (n=85) were not tested for antimicrobial susceptibility.

7.4.1 *E. faecalis* from pigs

Overall, 22% of the *E. faecalis* isolates from pigs were susceptible to all antimicrobials in the test panel, a level similar to that observed in 2021, the previous year of monitoring in pigs (Table 7.4).

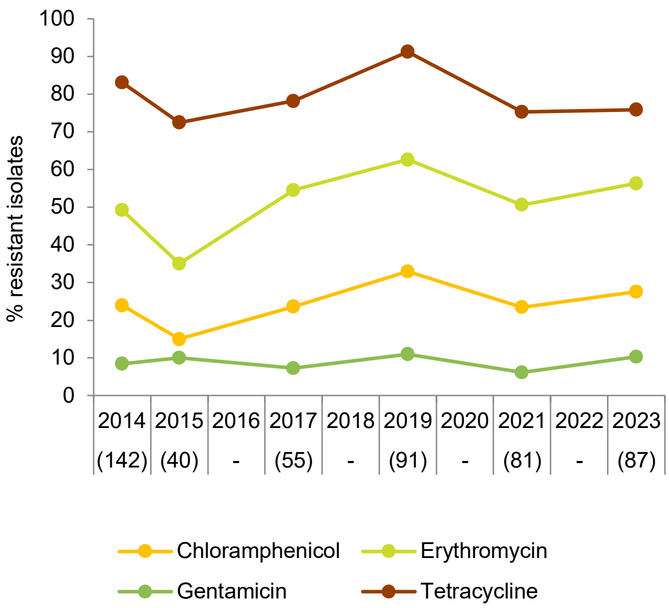
As in 2021, no *E. faecalis* isolates showed resistance to ampicillin, ciprofloxacin, linezolid, teicoplanin, tigecycline or vancomycin. Unlike 2021, in 2023, two isolates (2%) showed resistance to daptomycin. Similar low proportions of daptomycin-resistant isolates have been occasionally observed in previous years (2010, 2014 and 2019). Resistance to tetracycline (76%) and erythromycin (56%), followed by resistance to chloramphenicol (28%) continued to be the most common, and resistance to gentamicin was also observed in 10% of the isolates. Notably, after the decrease observed in 2021, the proportion of resistance to each of these four substances has increased in 2023, by magnitudes of 1% (tetracycline), 4% (gentamicin) and 5% (chloramphenicol and erythromycin) (Figure 7.4).

Table 7.4 Resistance (%) in *Enterococcus faecalis* isolates from pigs, Denmark, 2023 DANMAP 2023

Antimicrobial agent	*Enterococcus faecalis* %
Ampicillin	0
Chloramphenicol	28
Ciprofloxacin	0
Daptomycin	2
Erythromycin	56
Gentamicin	10
Linezolid	0
Teicoplanin	0
Tetracycline	76
Tigecycline	0
Vancomycin	0
Fully sensitive (%)	22
Number of isolates	87

E. faecalis are assumed inherently resistant to streptogramins (Quinopristin/Dalfopristin)

Figure 7.4 Resistance (%) among *Enterococcus faecalis* isolates from pigs, Denmark, 2023 DANMAP 2023



Number of isolates included each year is presented in parentheses

Among the resistant *E. faecalis* (N=65), four different multidrug-resistance profiles were observed in 29 isolates (33%). Combined resistance to chloramphenicol, tetracycline and erythromycin was the most common (22%; 19 isolates), followed by additional resistance to gentamicin (6%; 5 isolates). Four isolates (5%) were resistant to gentamicin, erythromycin and tetracycline, and a single isolate (1%) was resistant to erythromycin, tetracycline and daptomycin.

7.4.2 Perspectives

Enterococci are commensal gut bacteria in both animals and humans and can occasionally cause human disease. In Denmark, most human infections are caused by *E. faecalis* and *E. faecium*. While invasive infections by *E. faecium* in humans have seen a marked decrease in 2022 and 2023 after an increasing trend, those caused by *E. faecalis* have been relatively more constant over the last decade, and suffered a more modest decrease in the past two monitoring years [Chapter 8, Section 8.2.5].

Monitoring of resistance in *E. faecalis* from pigs has occurred in odd years since 2015. In contrast to the decrease observed in 2021, resistance levels in 2023 have overall increased (Figure 7.4).

In 2023, *E. faecalis* isolates recovered from pigs exhibited no resistance to ampicillin or vancomycin, antimicrobials often used to treat complicated infections in humans caused by enterococci. Furthermore, the isolates showed no resistance to linezolid and very low occurrence of resistance to daptomycin (Table 7.4). Both substances are used to treat multidrug-, vancomycin-resistant enterococci infections.

Given the resistance patterns observed in 2023 in *E. faecalis* from pigs and those causing invasive infections in humans, there is no strong suggestion that pigs are a potential zoonotic origin for the selected human isolates. However, the zoonotic nature of enterococci cannot be assessed solely based on antimicrobial resistance profiles. Recently, a phylogenetic study with Danish *E. faecium* isolates showed an almost absent overlap between animal- and food-associated strains and the hospital-associated clade of human isolates, while the overlap was far more extensive with the community-associated *E. faecium* [Roer et al., 2024. Microbiol Spectr 12:e03724-23.]. This again suggests that zoonotic transmission of enterococci may occur, but does not seem to be associated with the strains causing serious infections in humans.

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