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 prevalence of fully sensitive **indicator *E. coli*** isolates from broilers, cattle or pigs. Nonetheless, in the last two monitoring years, there was a steady increase in fully sensitive *E. coli* from broilers and a steady decrease in fully sensitive *E. coli* from cattle.

As in previous years, no amikacin, colistin, meropenem or tigecycline resistance were detected in indicator *E. coli*. Resistance to ciprofloxacin continued to be low in cattle and pigs and increased in broilers, reaching the maximum prevalence of the last 10 years (18%). Similarly to 2021, in 2022 azithromycin resistance was detected in a small number of isolates from pigs (3%), while the occurrence of chloramphenicol resistance decreased (8%).

The relative occurrence of multidrug-resistant indicator *E. coli* compared to the previous year decreased in broilers and pigs, and increased in cattle. Combined resistance to ampicillin, sulfamethoxazole, and tetracycline (ASuT) continued to be the most common multidrug-resistance profile. However, the relative occurrence of other multidrug-resistance profiles has increased in isolates from broilers and cattle in the past 5 years.

Importantly, as in previous years, samples from broilers and from broiler meat examined for **carbapenemase-producing (CP) *E. coli*** (including OXA-48) were found negative. Similarly, CP-producing *E. coli* was not detected in turkey meat in 2022.

The occurrence of **beta-lactamase-producing *E. coli***, obtained through selective procedures, continued the decreasing trend observed since 2018 in broilers and broiler meat. In 2022, imported turkey meat presented an occurrence of 52%. Antimicrobial susceptibility testing of ESBL/AmpC-producing *E. coli* from broilers showed an increase in resistance to fourth generation cephalosporins (cefepime), and a single isolate was found resistant to ertapenem. Resistance to ciprofloxacin was abundantly found in isolates from imported broiler meat (100%) and imported turkey meat (83%). Azithromycin resistance was also found in single isolates from those samples and isolates from imported turkey meat were also somewhat resistant to colistin (7%), ertapenem (2%) and gentamicin (10%).

Whole genome sequencing of beta-lactamase-producing *E. coli* revealed ESBL, AmpC and ESBL+AmpC genotypes. All AmpC genotypes encoded upregulated AmpC promotor C-42T mutations. The plasmid-mediated CMY-2 gene was observed in ESBL+AmpC genotypes. Among the ESBL genotypes, 14 different ESBL genes were detected, with most variation in isolates from imported turkey meat. The latter presented a high frequency of CTX-M-15 and 46% had more than one ESBL encoding gene.

In 2022, 39% of ***E. faecalis*** and 52% of ***E. faecium*** isolated from broilers were fully sensitive. None of the enterococci isolates showed resistance to ampicillin, chloramphenicol, daptomycin, gentamicin, linezolid, teicoplanin, tigecycline or vancomycin. Combined resistance to tetracycline and erythromycin continued to be among the most common resistance profiles.

7.1 Introduction

Escherichia coli and *Enterococcus* are included in the DANMAP programme to monitor the occurrence of antimicrobial resistance 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], while others found no evidence of transmission between animals and the general human population [Dorado-Garcia et al 2018, J Antimicrob Chemother, 73: 339-347; Findlay et al 2020, Applied and Environmental Microbiology, 87(1): e01842-20]. 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. In recent years, CPE 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 2023, EFSA Journal 2023; 21(3):7867].

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 EU harmonised monitoring of antimicrobial resistance [Decision 2020/1729/EU]. In 2022, isolates were obtained from randomly selected caecal samples collected from healthy broilers, cattle, and 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 interpretations are presented in Chapter 10.

7.2 Indicator *Escherichia coli*

Indicator *E. coli* isolates were obtained from 96% of caecal samples from broilers (195/204), 94% of samples from pigs (176/188) and 96% of samples from cattle (112/117). These isolates were obtained with a non-selective isolation procedure. Results obtained by selective procedures for specific monitoring of ESC- and CP-producing *E. coli* are presented in Section 7.3.

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, cattle or pigs during the past five years of monitoring (Figure 7.2) (p-values of 0.09 for broilers, 0.16 for cattle and 0.33 for pigs). Nevertheless, the percentage of broiler isolates susceptible to all antimicrobials in the test panel has increased in the past two years, from 58% in 2020 to 67% in 2022. In contrast, the percentage of cattle isolates fully sensitive has decreased from 91% in 2020 to 84% in 2022 (Table 7.1).

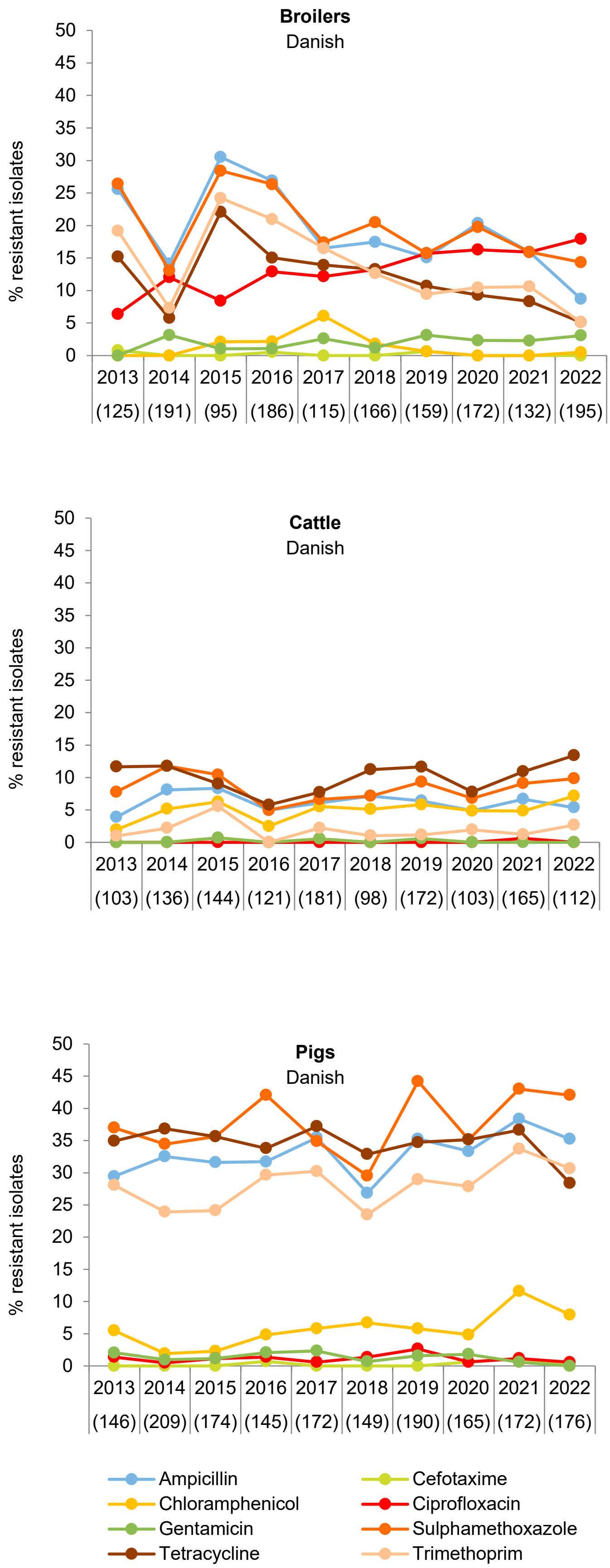
Compared to 2021, the occurrence of resistance to most antimicrobials in the test panel suffered mostly minor fluctuations (1 to 2%). A few exceptions were found in isolates from broilers, where resistance to ampicillin, tetracycline and trimethoprim decreased by 7%, 3% and 6%, respectively, while resistance to (fluoro)quinolones increased (by 4% for nalidixic acid and 2% for ciprofloxacin). In isolates from pigs, resistance to ampicillin, sulfamethoxazole, tetracycline and trimethoprim also decreased (by 9% for tetracycline and by 3% for the other three substances), as well as resistance to chloramphenicol, which decreased by 4%. These decreases contradict the previously observed increase in 2021 among isolates from pigs. In contrast, the percentage of *E. coli* from cattle resistant to ampicillin, sulfamethoxazole, tetracycline, trimethoprim and chloramphenicol all increased in 2022, although only by magnitudes of 1% to 2% (Figure 7.1).

Table 7.1 Resistance (%) in *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2022

	Broilers	Cattle	Pigs
Antimicrobial agent	Danish %	Danish %	Danish %
Amikacin	0	0	0
Ampicillin	9	5	35
Azithromycin	0	0	3
Cefotaxime	0	0	0
Ceftazidime	0	0	0
Chloramphenicol	<1	7	8
Ciprofloxacin	18	0	<1
Colistin	0	0	0
Gentamicin	3	0	0
Meropenem	0	0	0
Nalidixic acid	18	0	0
Sulfamethoxazole	14	10	42
Tetracycline	5	13	28
Tigecycline	0	0	0
Trimethoprim	5	3	31
Fully sensitive (%)	67	84	49
Number of isolates	195	112	176

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

Figure 7.1 Resistance (%) among *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2022



The number of isolates included each year is shown in parentheses

As in previous years, no isolates resistant to amikacin, colistin, meropenem or tigecycline were detected. Additionally, in 2022, resistance to 3rd generation cephalosporins was not detected in indicator *E. coli* using non-selective methods. Azithromycin resistance was, similarly to 2021, detected in 3% of the isolates from pigs (Table 7.1).

Resistance to (fluoro)quinolones continues to be low (<1%) or not observed in *E. coli* from cattle and pigs, but it increased to 18% in 2022 among broiler isolates, which gives continuity to an ongoing increasing trend (Figure 7.1). While this trend is not significant over the past 5-years (p-value=0.131), a significant increasing trend was determined for the past 10-years of monitoring (p-value=0.0004).

Compared to the two previous years, the occurrence of resistant and multidrug-resistant (MDR) *E. coli* isolates continued to decrease in broilers and to increase in cattle. In pigs, resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT) appears to be relatively stable, and annual oscillations in the occurrence of other MDR profiles occur, with no apparent ongoing increasing or decreasing trend (Figure 7.2). ASuT resistance was again the predominant MDR profile in isolates from pigs, and occurred in half of the MDR isolates from cattle and in 20% (2 out of 8 isolates) of the MDR isolates from broilers.

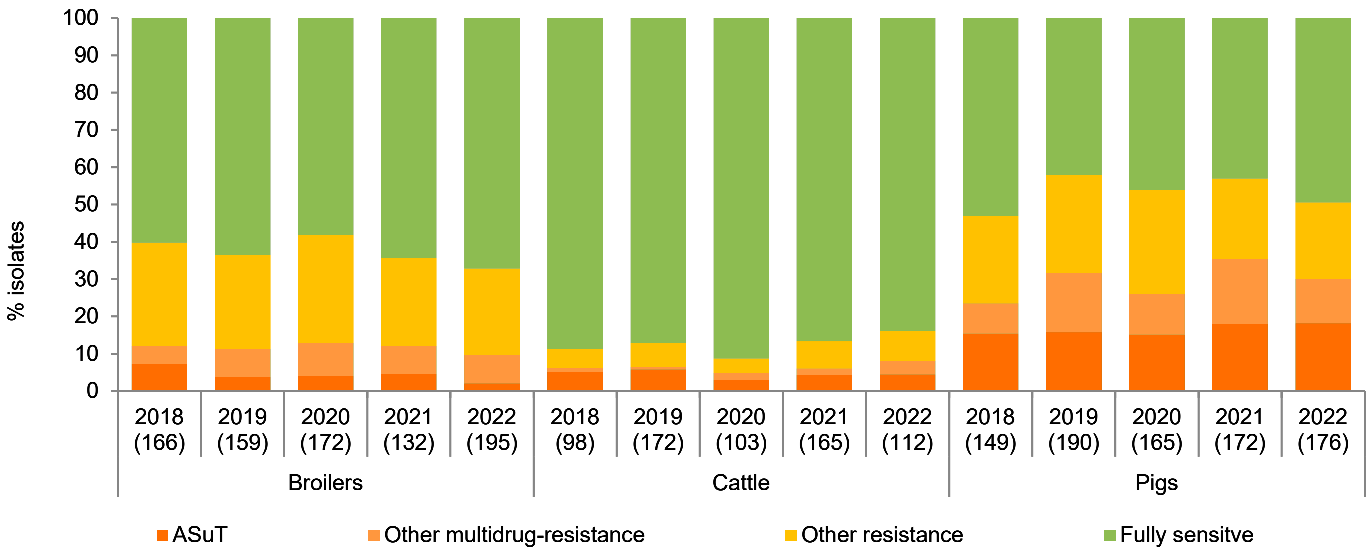
Among indicator *E. coli* isolated with a non-selective procedure, no presumptive ESBL-producing isolates were found (Table 7.1).

7.2.2 Perspectives

At the EU level, full sensitivity in indicator *E. coli* isolated from pigs and cattle has not changed significantly between 2015 and 2021. Significant decreasing trends in antimicrobial resistance have been observed for some countries in isolates from pigs and from cattle. Also at the EU level, full sensitivity in indicator *E. coli* from broilers has significantly increased between 2014 and 2020, with several countries showing this significant trend at the individual level. No significant increasing or decreasing trends in full sensitivity were reported for Denmark, individually, in the European Summary Report [EFSA/ECDC 2023, EFSA Journal 2023; 21(3):7867].

Accordingly, and as in previous years, in DANMAP 2022 no significant increasing or decreasing trends were observed in the occurrence of fully sensitive indicator *E. coli* recovered from broilers, cattle or pigs in the last 5-year monitoring period (in DANMAP 2022, between 2018 and 2022). It is possible that different trends are observed when different monitoring periods are included in the analysis, different statistical methods are applied or data are aggregated at different levels (annually, monthly, etc). In DANMAP 2022, an alternative approach was applied to perform a trend analysis of the occurrence of resistance in indicator *E. coli* (see Textbox 7.1).

Figure 7.2 Resistance (%) among *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2022



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

Furthermore, at the EU level, a clear negative association has been determined between the probability of full sensitivity in indicator *E. coli* and the overall consumption of antimicrobials by food-producing animals [EFSA/ECDC/EMA 2021, JIACRA III, DOI 10.2900/056892]. That analysis was based on monitoring data of total antimicrobial use and the percentage of full sensitivity in isolates from broilers, turkeys, pigs, and veal calves, from more than 20 countries, with a large variation in consumption and resistance levels among them. In the future, the association between antimicrobial use and full sensitivity of indicator *E. coli* should be assessed separately for broilers, pigs and cattle, and specifically for Denmark.

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

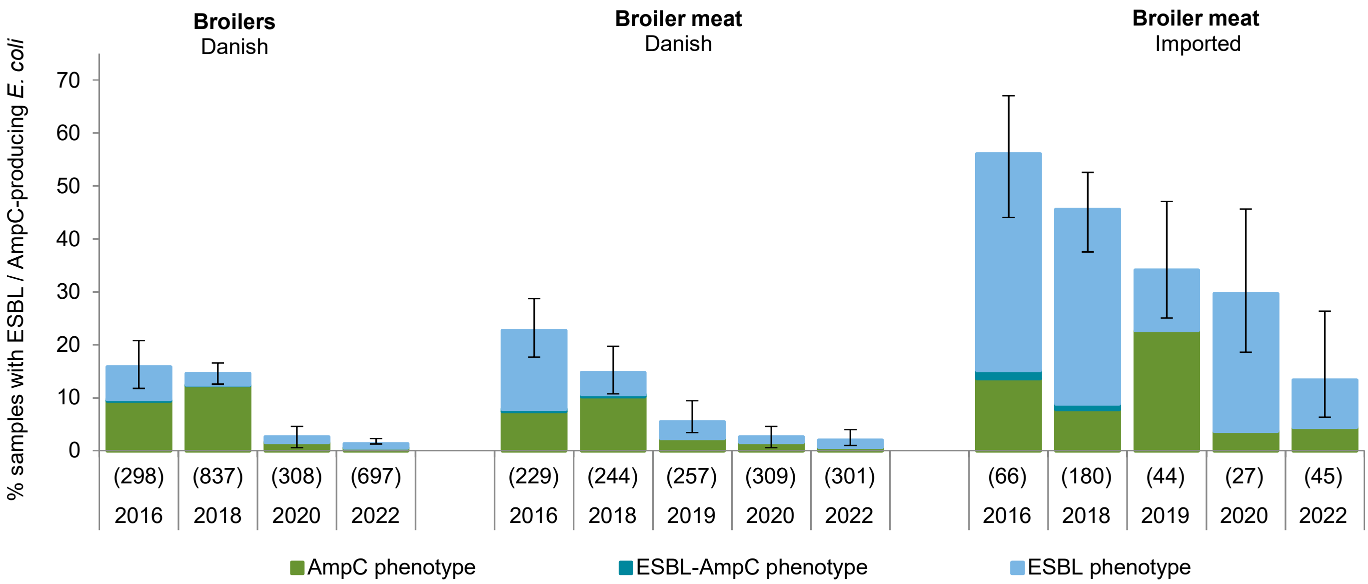
In 2022, caecal samples collected from broilers at slaughter, and from packages of fresh, chilled broiler- and turkey meat collected from Danish wholesale and retail outlets were monitored for the presence of extended-spectrum beta-lactamase (ESBL)-, cephalosporinase (AmpC)-, and carbapenemase (CP)-producing *E. coli*. In accordance with the harmonised EU monitoring, packages of meat were collected at retail without pre-selecting by country of origin, hence including in principle both imported and domestically-produced products. Of the samples randomly collected at retail, 13% of broiler meat and 100% of turkey meat were imported products. According to the new Decision 2020/1729/EU, monitoring of turkey meat for ESBL-, AmpC-, and CP-producing *E. coli* is mandatory in even years.

Using selective procedures, ESBL/AmpC-producing isolates were obtained from broilers (9/697 (1%) flocks), broiler meat (Danish: 6/301 (2%) samples, imported: 6/45 (13%) samples), and imported turkey meat (59/113 (52%) samples). The selective procedures for detection of CP-producing *E. coli* (including oxacillinase-producing OXA-48-like enzymes), recovered no isolates (Table 7.2).

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

Following selective enrichment, *E. coli* resistant to 3rd generation cephalosporins (cefotaxime and/or ceftazidime) were obtained from 1% (CI 95%: 1-2%) of samples from broilers, 2% (CI 95%: 1-4%) of samples from Danish broiler meat, 13% (CI 95%: 6-26%) of samples from imported broiler meat (from three different countries) (Figure 7.3), and 52% (CI 95%: 43-61%) of samples from imported turkey meat (from two different countries, 98% from a single country). In 2022, the prevalence of ESBL/AmpC-producing *E. coli* continued to decrease in isolates from broilers and broiler meat. Furthermore, as observed in the last four monitoring years, the occurrence of ESBL/AmpC-producing *E. coli* continues to be higher in imported broiler meat compared to Danish broiler meat (Figure 7.3). In 2022, the relative frequency of ESBL-producing and AmpC-producing phenotypes remained mostly unchanged in comparison to 2020, except among isolates from imported broiler meat, which showed an increase in the relative occurrence of the AmpC-producing phenotype (from 13% to 33%). In isolates recovered from imported turkey meat, the ESBL-producing phenotype was clearly predominant (90%) (Table 7.2).

Figure 7.3 Occurrence (%) of samples with phenotypic ESBL- or AmpC-producing *E. coli* from animals and meat recovered by selective enrichment, Denmark 2016-2022
DANMAP 2022



Number of samples tested per year is presented in parentheses. Classification of ESBL and AmpC phenotypes is based on the MIC results (Materials and Methods, Section 10.7.2). Confidence intervals for total proportion of samples positive for a phenotype calculated as 95% binomial proportion Wilson intervals

All the recovered ESBL/AmpC-producing isolates from broilers and Danish broiler meat were resistant to both 3rd generation cephalosporins (cefotaxime and ceftazidime) and ampicillin. Isolates from imported broiler- and turkey meat were also 100% resistant to cefotaxime and to ampicillin, but 83% and 95% resistant to ceftazidime, respectively. In contrast, resistance to 4th generation cephalosporins (cefepime) was found at a higher prevalence in imported broiler meat (83%) and imported turkey meat (95%), when compared to Danish broilers and broiler meat (67%) (Table 7.2). Compared to 2020, the levels of resistance to cefepime increased by 17% in isolates from domestic broilers and broiler meat, and decreased by 17% in isolates from imported broiler meat.

During the same period, the observed resistance to fluoroquinolones (ciprofloxacin) has markedly decreased in ESBL/AmpC-producing *E. coli* from Danish broilers (88% to 44%) and from Danish broiler meat (63% to 17%). In contrast, resistance markedly increased in isolates from imported broiler meat (50% to 100%). In 2022, ciprofloxacin resistance was also observed in 83% of the isolates from imported turkey meat. As in 2020, no resistance to colistin, meropenem or imipenem was observed in the specific monitoring of ESBL/AmpC-producing *E. coli* from broilers and broiler meat in 2022. However, ertapenem resistance was observed in one out of 11 isolates (11%) from broilers and one out of 59 isolates from imported turkey meat (2%), while colistin-resistant ESBL/AmpC-producing *E. coli* were observed in 7% of the turkey meat samples. Azithromycin resistance was observed in one out of six isolates from imported broiler meat (17%) and in a single isolate from imported turkey meat (2%), while gentamicin resistance was only detected among isolates from imported turkey meat (10%). As in previous years, resistance to tigecycline and temocillin was not observed among the isolates collected in 2022 (Table 7.2).

The genetic basis for ESBL- and AmpC enzymes was detected in most isolates recovered by selective enrichment. The detected enzymes corresponded to the phenotypes derived from the susceptibility testing for the majority of the isolates. In 5 isolates (2 from broiler meat and 3 from broilers), whole genome sequencing revealed both ESBL and AmpC encoding genes, even though the susceptibility testing showed a AmpC-producing phenotype (Tables 7.2 and 7.3). Three isolates from turkey meat showed an ESBL and AmpC producing phenotype however, only ESBL-encoding genes were detected.

Among the AmpC-producing isolates recovered in 2022, resistance was mainly conferred by upregulated AmpC promotor C-42T mutations (one isolate from broilers, one from Danish broiler meat and three from imported turkey meat). The CMY-2 plasmid-mediated AmpC enzyme was detected in a single AmpC-producing *E. coli* from imported broiler meat (Table 7.3).

Among all ESBL-producing isolates, 14 different ESBL genes were detected, of which 9 occurred as the only encoding gene (CTX-M-1, CTX-M-14, CTX-M-15, CTX-M-27, CTX-M-32, CTX-M-55, SHV-12, TEM-1B and TEM-52B). Overall, the most commonly observed ESBL encoding genes across isolates from broilers, broiler meat and turkey meat were CTX-M-1 and TEM-1B. Notably, the encoding gene CTX-M-15 was highly frequent in isolates from imported turkey meat only (Table 7.3). Furthermore, in isolates from imported turkey meat, 13 out of the total 14 different detected ESBL genes were observed, and 46% of the isolates had more than one ESBL encoding gene, with two isolates harbouring three different genes (SHV-12/TEM-1B/TEM-1D and SHV-12/TEM-1B/TEM-135) (Table 7.3).

Among isolates that harboured both ESBL and AmpC encoding genes, upregulated AmpC promotor C-42T mutation was detected in two isolates from broilers. ESBL- and AmpC-producing isolates harbouring the AmpC plasmid-mediated CMY-2 were observed in single isolates from Danish broilers and broiler meat, and in two isolates from imported broiler meat. All ESBL and AmpC genotypes detected in 2022 were due to the presence of the ESBL encoding gene TEM-1B. No ESBL and AmpC genotypes were detected in isolates from imported turkey meat (Table 7.3).

In total, 35 MLSTs were observed among all ESBL/AmpC-producing *E. coli* isolates. The most common MLSTs were ST1001 in Danish broiler meat, ST6448 in imported broiler meat, ST4663 in broilers, and ST4981 in imported turkey meat. The isolates that harboured the CMY-2 genotype were attributed to ST1001 (Danish broiler meat), ST2473 (broilers), ST155 and ST1112 (imported broiler meat).

7.3.2 Perspectives

An obvious reduction in the ESBL/AmpC-producing *E. coli* in Danish broilers and broiler meat has occurred since 2018 (Figure 7.3). This reduction is likely the result of the requirement that imported breeding and production animals must be tested and found negative for ESBL/AmpC-producing *E. coli* before they are allowed into the country.

Table 7.2 Resistance (%) and beta-lactam resistance phenotype distribution in ESBL/AmpC-producing *Escherichia coli* recovered by selective enrichment from animals and meat, Denmark DANMAP 2022

	Broilers	Broiler meat		Turkey meat
Antimicrobial agent	Danish %	Danish %	Import %	Import %
Amikacin	0	0	0	0
Ampicillin	100	100	100	100
Azithromycin	0	0	17	2
Cefepime	67	67	83	95
Cefotaxime	100	100	100	100
Cefotaxime/clavulanic acid	44	33	33	5
Cefoxitin	44	33	33	10
Ceftazidime	100	100	83	95
Ceftazidime/clavulanic acid	44	33	33	5
Chloramphenicol	0	17	50	38
Ciprofloxacin	44	17	100	83
Colistin	0	0	0	7
Ertapenem	11	0	0	2
Gentamicin	0	0	0	10
Imipenem	0	0	0	0
Meropenem	0	0	0	0
Nalidixic acid	44	17	83	58
Sulfamethoxazole	0	17	100	58
Temocillin	0	0	0	0
Tetracycline	11	17	83	58
Tigecycline	0	0	0	0
Trimethoprim	11	0	83	43
Number of AmpC phenotypes	4	2	2	3
Number of ESBL phenotypes	5	4	4	53
Number of ESBL+AmpC phenotypes	0	0	0	3
Number of isolates (%)	9 (1%)	6 (2%)	6 (13%)	59 (52%)
Number of samples	697	307	45	113

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

At the EU-level, there is a large variation in the prevalence of ESC-producing *E. coli* recovered from animals and meat. In 2020, prevalence ranged between 0.3% and 97% in broilers and broiler meat and between 0% and 70.4% in fattening turkeys [EFSA/ECDC 2022. EFSA Journal 2022;20(3):7209]. With the prevalence levels observed in 2022 (1% to 2%), Denmark continues to be among the countries with a lower occurrence of beta-lactamase-producing *E. coli* in domestic broilers and meat thereof. The enzymes of the ESBL and AmpC-producing *E. coli* in broilers and broiler meat seem to be consistent with the enzymes detected in previous years, but their attribution to different MLSTs suggests the occurrence of horizontal gene transfer.

This was the first year of mandatory monitoring of ESBL/AmpC-producing *E. coli* in turkey meat, according to Decision 2020/1729/EU. Results have shown a relatively high occurrence of ESBL/AmpC-producing *E. coli* in imported turkey meat when compared to imported broiler meat. Additionally, there was a large variety of ESBL enzymes detected in isolates from turkey meat, among which the most frequently detected (CTX-M-15) is also often encountered in isolates from bloodstream infections in humans (see Chapter 3). The public health relevance of these findings can be best assessed after some years of continuous harmonized monitoring.

The zoonotic transmission of beta-lactamase-producing *E. coli* continues to be investigated, with studies presenting different conclusions. In Chapter 3, ESBL/AmpC-producing *E. coli* isolates collected in Denmark between 2018 and 2022 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 the 1162 samples tested in 2022. In 2020 and 2021, in the EU-monitoring of CP-producing *E. coli*, 14 and 29 isolates were detected with phenotypic and genotypic methods, respectively. Those included in total only four isolates from broilers and one from fattening turkeys [EFSA/ECDC 2022. EFSA Journal 2022;20(3):7209]. However, 2022 was the first year of mandatory monitoring of CP-producing *E. coli* in poultry, according to Decision 2020/1729/EU. Thus, it is possible that a higher number of isolates will be reported 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 DANMAP 2022

	Broilers	Broiler meat		Turkey meat
Enzymes	Danish	Danish	Import	Import
CTX-M-1	2	1		3
CTX-M-14				2
CTX-M-15				28
CTX-M-27				6
CTX-M-32				2
CTX-M-55			2	5
CTX-M-65			1	1
OXA-1				2
SHV-12		1		6
TEM-135				3
TEM-176				1
TEM-1B	3	1	4	21
TEM-1D				1
TEM-52B	1	2		
CMY-2	1	1	2	
Chromossomal AmpC (C-42T)	3	1		3
Number of AmpC genotypes	1	1	1	3
Number of ESBL genotypes (two or more enzymes)	3 (0)	4 (0)	4 (2)	54 (25*)
Number of AmpC+ESBL genotypes	3	1	1	0
Not available	2	0	0	2
Number of ESC isolates (%)	9 (1%)	6 (2%)	6 (13%)	59 (52%)
Number of samples	697	307	45	113

Number (%) positive samples are isolates recovered by selective enrichment methods for specific 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). Not available refers to isolates without available WGS results. * Two of the 25 isolates from turkey meat with more than one ESBL enzyme had three different enzymes

7.4 Indicator Enterococci

Enterococci were obtained from 331 (99%) of the 336 faecal samples taken from broiler flocks at slaughter, and antimicrobial susceptibility testing was performed on 28 *E. faecalis* and 305 *E. faecium* isolates.

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

Overall, 39% of the *E. faecalis* and 52% of the *E. faecium* isolates from broilers were susceptible to all antimicrobials in the test panel (Table 7.4).

As in 2020, the previous year of monitoring in broilers, no enterococci isolates showed resistance to ampicillin, linezolid, gentamicin, teicoplanin, tigecycline or vancomycin. Additionally, in 2022, no isolates showed resistance to chloramphenicol or daptomycin. Resistance to tetracycline and erythromycin continued to be the most common.

Among *E. faecalis* isolates, compared to 2020, the occurrence of erythromycin resistance increased from 38% to 43%, while the occurrence of tetracycline resistance decreased from 62% to 50%. Resistance of *E. faecium* isolates to ciprofloxacin, erythromycin and tetracycline was at the same level as in 2020 (Table 7.4 and Figure 7.4).

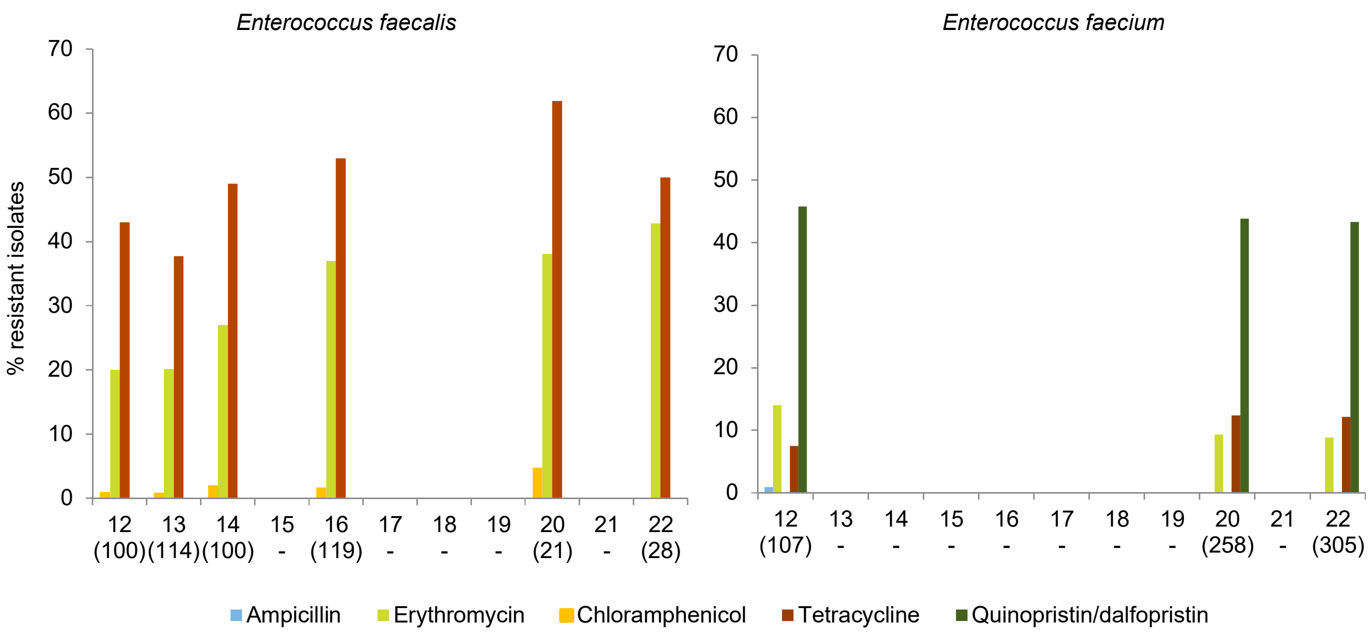
Among the resistant *E. faecalis* (N=17), three different resistance profiles were observed. Combined resistance to tetracycline and erythromycin was the most common (9 isolates) however, isolates resistant to only tetracycline (N=5) or erythromycin (N=3) were also observed. Among the resistant *E. faecium* isolates (N=147) four different resistance profiles were observed, with 102 isolates showing resistance to a single antimicrobial, 33 showing resistance to two substances, and 12 showing multidrug-resistance. The most common profile was resistance to quinopristin-dalfopristin (N=92), followed by combined resistance to quinopristin-dalfopristin and tetracycline (N=16), and by multidrug-resistance to tetracycline, erythromycin and quinopristin/dalfopristin (N=11). One *E. faecium* isolate was resistant to all four antimicrobials.

Table 7.4 Resistance (%) in Enterococci isolates from broilers, Denmark DANMAP 2022

Antimicrobial agent	*Enterococcus faecalis* %	*Enterococcus faecium* %
Ampicillin	0	0
Chloramphenicol	0	0
Ciprofloxacin	0	3
Daptomycin	0	0
Erythromycin	43	9
Gentamicin	0	0
Linezolid	0	0
Quinupristin/dalfopristin	-	43
Teicoplanin	0	0
Tetracycline	50	12
Tigecycline	0	0
Vancomycin	0	0
Fully sensitive (%)	39	52
Number of isolates	28	305

E. faecalis are assumed inherently resistant to streptogramins (Quinupristin/dalfopristin)

Figure 7.4 Resistance (%) among Enterococci isolates from broilers, Denmark 2012-2022 DANMAP 2022



Number of isolates included each year is presented in parentheses

7.4.2 Perspectives

Enterococci are commensal gut bacteria in both animals and humans, and can occasionally cause human disease. In Denmark, the majority of human infections are caused by *E. faecalis* and *E. faecium*, and a 5.8% increase in invasive infections caused by these two species was observed between 2013 and 2022 [Chapter 8, Section 8.2.5].

In 2022, *E. faecalis* and *E. faecium* isolates recovered from broilers exhibited no resistance to ampicillin, gentamicin or vancomycin, antimicrobials often used to treat complicated infections in humans caused by enterococci. Furthermore, isolates showed no resistance to linezolid or daptomycin, which are used to treat multidrug-resistant vancomycin-resistant enterococci infections (Table 7.4).

Monitoring of enterococci in animals has not been constant in DANMAP. Nevertheless, compared to previous monitoring years, occurrence of resistance in *E. faecium* in broilers seems relatively constant, while resistance in *E. faecalis* has increased since 2012 (Figure 7.4).

Compared to *E. faecalis*, *E. faecium* recovered from broilers exhibited lower occurrence of resistance. Among enterococci recovered from humans, the occurrence of resistance is generally higher in *E. faecium* than in *E. faecalis* [Chapter 8, Section 8.2.5].

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Textbox 7.1

Antimicrobial resistance trends in indicator *E. coli* in Danish pigs, broilers, and cattle 2014 to 2022

Background

Since 2014, the monitoring of antimicrobial resistance (AMR) in indicator *E. coli* isolated from food-producing animals (pigs, broilers, and cattle) has been mandatory by EU legislation.

Although surveillance is essential to follow antimicrobial resistance trends over time and to determine the effects of interventions such as reductions in antimicrobial use, the evaluation and optimization of AMR trend analysis are still needed [1]. To better understand the data collected through DANMAP on indicator *E. coli*, we investigated temporal trends and points in time when these trends show significant changes.

Materials and Methods

We aggregated data collected through DANMAP on monthly phenotypic resistance of indicator *E. coli* for the period 2014 – 2022 by food-producing animal species (broilers, pigs, and cattle). Each isolate was investigated for full sensitivity and for multidrug-resistance (as defined in DANMAP). We analysed the monthly variation in the proportion of fully sensitive and multidrug-resistant isolates for broilers, pigs, and cattle separately. Using linear weighted moving averages, we imputed missing values to ensure a continuous time series. After, we performed a seasonal trend decomposition using the locally weighted regression (LOESS) method to separate the time series into its trend, seasonal, and irregular components. Finally, we did a change-point analysis using the pruned exact linear time (PELT) method to detect any point in time at which a statistically significant change in the proportion of fully sensitive or multidrug-resistant indicator *E. coli* occurred.

Results and discussion

Data on antimicrobial susceptibility testing collected between 2014 and 2022 were available for 1,411, 1,552, and 1,232 indicator *E. coli* isolates from broilers, pigs, and cattle, respectively. The estimated monthly proportion of fully sensitive and multidrug-resistant isolates for each food-producing animal species is shown in Figures 1-3. Results show that the proportion of fully sensitive indicator *E. coli* is highest on average within the cattle population, whereas pigs show the lowest proportion. The trend lines (Figures 1–3) show a visual increase in the proportion of fully sensitive indicator *E. coli* isolates in broilers and pigs starting in the first quarter of 2016 and in the second quarter of the same year, respectively. This increase in *E. coli* from pigs can be associated with the implementation, in 2016, of the new Yellow Card for pigs with a focus on reducing the use of critically important antimicrobials. In contrast, a decreasing trend can be observed for cattle from July 2020. This coincided with an increase in the use of b-lactamase sensitive penicillins and amphenicols observed in calves in 2020 [2]. Variations in the proportion of fully sensitive isolates are accompanied by an inverse parallel trend in the proportion of multidrug-resistance for broilers. For pigs and cattle, these combined trend fluctuations are not so clear. Note however that proportions of isolates with resistance to only one or two substances were not considered.

Although we can visually detect these fluctuations in the proportion of fully sensitive and multidrug-resistant indicator *E. coli*, our results show no statistically significant changes over the time period studied. Still, in the future, these data can be explored with different aggregation levels, namely by antimicrobial class, and using a larger time period, which can possibly show different results. Furthermore, a multivariate time series analysis can be implemented where the associations between both phenotypic and genotypic AMR and AMU can be explored.

continued ... Textbox 7.1

Figure 1 Monthly proportion of fully sensitive and multidrug-resistant indicator *E. coli* isolated from broilers (n = 1,411), Denmark 2014-2022. The grey lines identify the time series trend calculated using the locally weighted regression (LOESS) method DANMAP 2022

Figure 2 Monthly proportion of fully sensitive and multidrug-resistant indicator *E. coli* isolated from pigs (n = 1,552), Denmark 2014-2022. The grey lines identify the time series trend calculated using the locally weighted regression (LOESS) method DANMAP 2022

Figure 3 Monthly proportion of fully sensitive and multidrug-resistant indicator *E. coli* isolated from cattle (n = 1,232), Denmark 2014-2022. The grey lines identify the time series trend calculated using the locally weighted regression (LOESS) method DANMAP 2022