7

RESISTANCE IN INDICATOR BACTERIA

7. Resistance in indicator bacteria

Highlights: In 2019, trends and levels of antimicrobial resistance in indicator *E. coli* from poultry, pigs and cattle were overall very similar to previous years.

The relative distribution of fully sensitive indicator *E. coli* from broilers (64%) and cattle (87%) was comparable to 2018, whereas a decrease from 53% to 42% was observed in fully sensitive isolates from pigs. Compared to 2018, a statistically significant increase (from 30% to 44%) in sulfonamide resistance and a moderate increase (from 23% to 32%) in occurrence of multidrug-resistance was observed in pig isolates. Among broiler and cattle isolates, the occurrence of multidrug-resistance was comparable to 2018 and only minor fluctuations (1%-2%) in resistance were observed between the years in cattle isolates. However, over the last 5-year period, the occurrence of multidrug-resistant *E. coli* isolates from broilers has decreased significantly (from 23% to 11%).

From broilers, only one isolate (<1%) resistant to cefotaxime and ceftazidime was detected by the non-selective method. More phenotypic resistant isolates were detected by the more sensitive selective isolation methods in samples from pigs and cattle, indicating that resistance was also present in a relatively small proportion of commensal *E. coli* in pigs and cattle as well. Furthermore, no colistin, meropenem and tigecycline resistance was detected. The slow but steady increase in resistance to ciprofloxacin/nalidixic acid in *E. coli* from broilers, observed over the last ten years, continued.

In 2019, selective isolation methods showed that trends of *E. coli* producing ESBL/AmpC in animals and meat aligned with the observations from 2015-2018: lower occurrence in cattle compared to pigs (8% vs. 27%), lower levels in domestically produced broiler meat and pork than in imported (5% and 3% vs. 34% and 42%, respectively), and the decreasing occurrence in Danish broiler meat continued. ESBL transferring enzymes often associated with human infections, such as CTX-M-1, CTX-M-14 and/or CTX-M-15 were detected in low or very low numbers in all sources, and chromosomal AmpC mutations in isolates from pigs and cattle were still dominant.

Importantly, again all samples examined for carbapenemase-producing *E. coli* (including OXA-48) were found negative.

In 2019, enterococci from pigs showed no resistance to vancomycin, linezolid, teicoplanin or tigecycline. The *E. faecalis* isolates were resistant to tetracycline (91%), erythromycin (63%) and chloramphenicol (33%) and compared to 2017, an increase in tetracycline (13%) chloramphenicol (9%) and erythromycin (8%) resistance has been observed. Resistance levels in *E. faecium* were comparable to the latest observations from 2010-2012 and the most commonly observed resistance was tetracycline (54%), erythromycin (20%) and ampicillin (12%).

Currently, the zoonotic risk linked to transfer of resistance to critically important antimicrobials from animals to humans appears to be very limited in Denmark.

7.1 Introduction

Escherichia coli and *Enterococcus* are included in the DANMAP programme to monitor occurrence of antimicrobial resistance in different reservoirs through 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 horisontal 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 production of extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases (AmpCs) is one of the fastest spreading antimicrobial resistance problems in both humans and production animals worldwide. Several studies report similar ESBL/AmpC genes, plasmids and/or clones of *E. coli* isolates in animals, meat and human infections, which suggests a zoonotic link [Roer et al 2019. J Antimicrob Chemother 74(3):557; Valcek et al 2019. J Antimicrob Chemother 74(8):2171].

Carbapenemase-producing Enterobactericeae (CPE) are a great threat to human health, because carbapenems are last-line antimicrobial drugs for treatment of infections caused by multidrug-resistant Gram-negative bacteria. Currently, CPE have been detected sporadically in production animals in EU but never in Denmark [EFSA/ECDC 2019. EFSA journal 17(2):5598].

Since 2014, isolation and antimicrobial susceptibility testing of indicator *E. coli*, extended-spectrum cephalosporinase- and carbapenemase-producing *E. coli* (ESC and CPE) has been performed according to the EU harmonised monitoring of antimicrobial resistance [Decision 2013/652/EU].

7.2 Indicator *Escherichia coli*

All isolates originated from caecal samples randomly collected from healthy pigs, broilers and cattle at slaughter. Only one isolate per farm was included. Susceptibility to the antimicrobials recommended by EFSA was measured by broth microdilution to determine minimal inhibitory concentrations (MIC). MIC distributions and occurrence of resistance among indicator *E. coli* are presented in the web annex (Table A7.1). These results were obtained using the non-selective isolation procedure. Results obtained by using selective procedures for detection of cefotaxime-resistant *E. coli* are reported in section 7.3.

7.2.1 Indicator *E. coli* from broilers, cattle and pigs

From 168 representative pools of broiler caeca collected at Danish slaughterhouses, 159 *E. coli* isolates were obtained and all 159 isolates were tested for antimicrobial resistance (Table 7.1). More than half (64%) of the broiler isolates were susceptible to all antimicrobials in the test panel. From 186 representative cattle caeca collected at Danish slaughterhouses, 174 *E. coli* isolates were obtained, and 172 of these were tested for antimicrobial resistance (Table 7.1). The vast majority of cattle isolates (87%) was susceptible to all tested antimicrobials. From 195 representative pig caeca collected at Danish slaughterhouses, 192 *E. coli* isolates were obtained and 190 of these were tested for antimicrobial resistance (Table 7.1). Less than the half (42%) of the isolates from pigs were susceptible to all antimicrobials tested.

Among isolates from all animals, no colistin, meropenem or tigecycline resistance was detected. Compared to 2018, a minor increase (13% to 16%) in ciprofloxacin and nalidixic acid (13% to 15%) resistance in broiler isolates was observed, whereas a minor decrease (4% to 1%) in azithromycin resistance was detected in pig isolates. Only one isolate from broilers was resistant to 3rd generation cephalosporins (cefotaxime and ceftazidime), whereas no resistance to these critically important antimicrobials was found in cattle or pigs using non-selective methods (Table 7.1).

Table 7.1 Resistance (%) in *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2019

	Broilers	Cattle	Pigs
Antimicrobial agent	Danish %	Danish %	Danish %
Ampicillin	15	6	35
Azithromycin	0	0	1
Cefotaxime	<1	0	0
Ceftazidime	<1	0	0
Chloramphenicol	<1	6	6
Ciprofloxacin	16	0	3
Colistin	0	0	0
Gentamicin	3	<1	2
Meropenem	0	0	0
Nalidixic acid	15	0	1
Sulfonamide	16	9	44
Tetracycline	11	12	35
Tigecycline	0	0	0
Trimethoprim	9	1	29
Fully sensitive (%)	64	87	42
Number of isolates	159	172	190

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel, and multidrug-resistant if resistant to 3 or more of the 12 antimicrobial classes included (Table 9.3)

Among pig isolates, a statistically significant increase (30% to 44%) in sulfonamide resistance was observed, whereas resistance levels to the other antimicrobials were comparable to the observations in 2018. (Figure 7.1). Compared to 2018, only minor fluctuations (1% to 2%) in occurrence of resistance were observed in isolates from cattle and broilers. The occurrence of ampicillin, sulfonamide, tetracycline and trimethoprim resistance was significantly higher in *E. coli* from pigs (35%, 44%, 35% and 29%, respectively) compared to isolates from cattle

(6%, 9%, 12% and 1%, respectively). Resistance to ampicillin and tetracycline was also significantly higher in isolates from pigs than from broilers (15% and 11%, respectively). In contrast, ciprofloxacin resistance was higher in isolates from broilers (16%) compared to isolates from cattle (0%) and pigs (3%).

The relative distribution of fully sensitive isolates from broilers and cattle in 2019 was almost identical to 2018 with a difference of 4% and 2%, respectively. Compared to 2018, a moderate decrease (53% to 42%) in prevalence of fully sensitive *E. coli* from pigs was observed (Figure 7.2). A moderate increase (23% to 32%) in multidrug-resistant isolates from pigs was detected, whereas the prevalence of multidrug-resistant isolates from broilers and cattle was almost identical, compared to 2018. Among the multidrug-resistant isolates, co-resistance to ampicillin, sulfonamide and tetracycline (ASuT) was commonly observed (Figure 7.2).

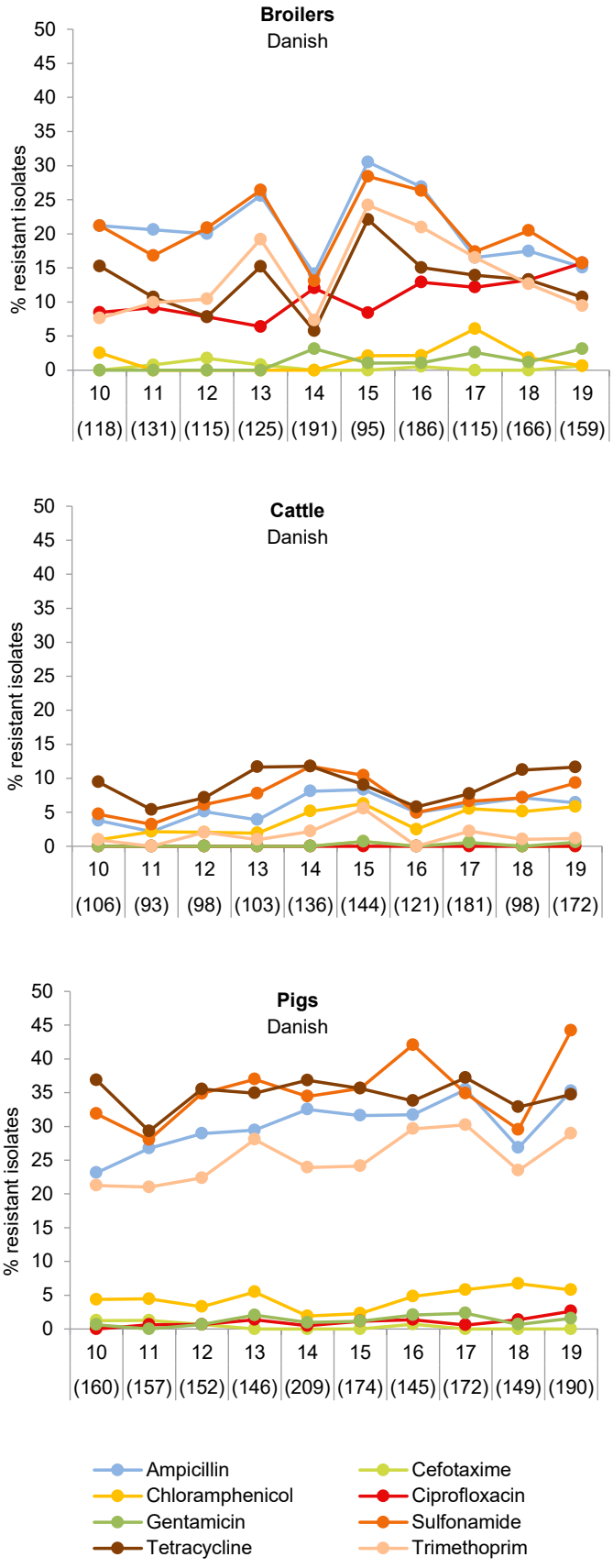
Over the last 5-year period, no statistically significant changes in the relative distribution of fully sensitive isolates from broilers, cattle or pigs have been observed, whereas a significant decrease in the relative occurrence of multidrug-resistant broiler isolates has occurred.

Among all resistant isolates from broilers (n = 58), 19 resistance profiles were observed. In total, 11% of all tested broiler isolates (n = 18) were classified as multidrug-resistant (resistance to ≥3 of the 12 antimicrobial classes in the *E. coli* test panel) and had 8 different resistance profiles (see web annex Table A7.2). The most common profiles included resistance to: i) ciprofloxacin and nalidixic acid, ii) ampicillin, sulfonamide and trimethoprim and iii) tetracycline; and were detected among 26%, 16% and 10% of resistant isolates from broilers, respectively. Noteworthy, a single ESBL-producing isolate with resistance to ampicillin, ceftazidime, cefotaxime, cefepime, sulfonamide and tetracycline was observed in the 2019 monitoring.

A total of, 10 resistance profiles were observed among resistant isolates from cattle (n = 22), and of these isolates, 11 were categorised as multidrug-resistant (see web annex Table A7.2). As in 2018, a high prevalence of tetracycline resistance was detected. The most common profiles included resistance to: i) ampicillin, chloramphenicol, sulfonamide and tetracycline, ii) sulfonamide and tetracycline and iii) tetracycline; and were observed among 36%, 18% and 14% of the resistant isolates from cattle, respectively.

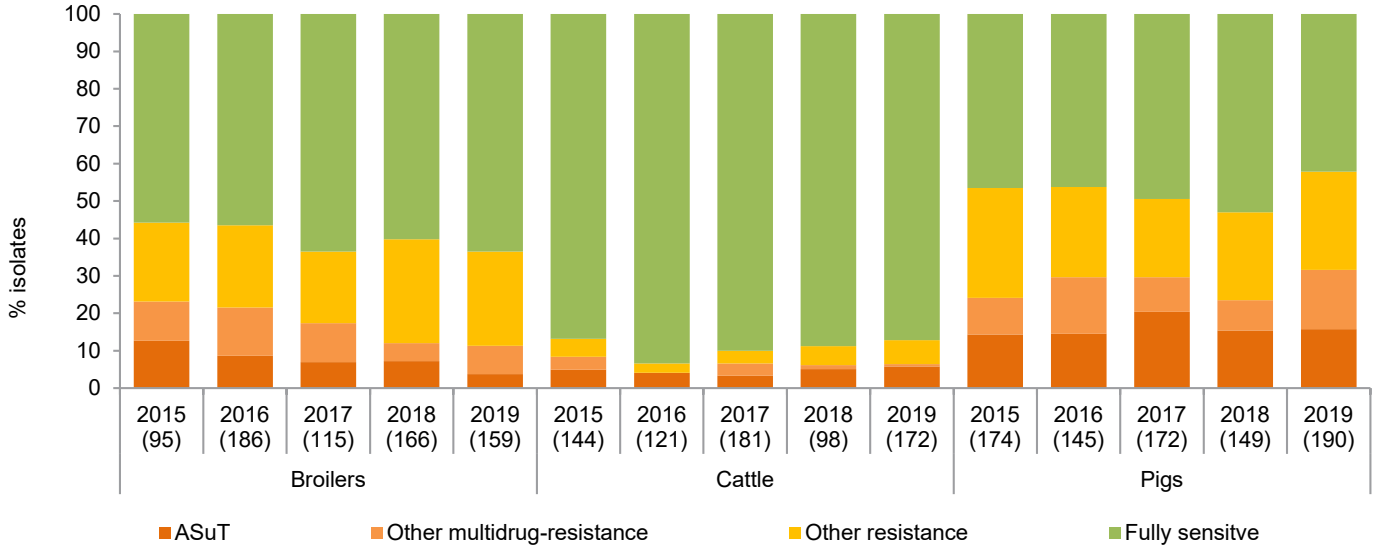
From pigs, a total of 28 resistance profiles were observed among all resistant isolates (n = 110), and 55% of these (n = 60) were categorised as multidrug-resistant (see web annex Table A7.2). Nineteen (17%) of all resistant isolates exhibited resistance to one type of antimicrobial only. Thus, 11%, 5%, 1% and 1% of the resistant isolates showed resistance to tetracycline, sulfonamide, trimethoprim and ampicillin, respectively.

Figure 7.1 Resistance (%) among *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2019



Note: Number of isolates included each year is presented in the parenthesis

Figure 7.2 Distribution (%) of multidrug-resistant, resistant and fully sensitive *Escherichia coli* isolates from broilers, cattle and pigs, Denmark
DANMAP 2019



Note: 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 3 or more of the 12 antimicrobial classes included (Table 9.3). ASuT are the multidrug-resistant isolates resistant to ampicillin, sulfonamide and tetracycline, but may also be resistant to other antimicrobials

Over the last 10-year period, there has been a slow but statistically significant increase in resistance to chloramphenicol, nalidixic acid, ciprofloxacin and gentamicin among broiler isolates. For pig isolates, a slow but statistically significant increase in resistance to ampicillin and trimethoprim was observed whereas a statistically significant increase in resistance to ampicillin and chloramphenicol was detected for cattle isolates, over the last 10-year period. Over the last 5-year period, no statistically significant increase in occurrence of resistance was observed among any of the animal isolates.

7.2.2 Perspectives

Antimicrobial resistance monitoring in commensal *E. coli* is considered a useful indicator of the selective pressure exerted by antimicrobial use on the intestinal microbiota of food-producing animals.

From a European perspective, based on the last published data from 2017 and 2018, indicator *E. coli* from Danish broilers and calves <1 year show noticeably low occurrence of resistance to any antimicrobial compared to the indicator *E. coli* from other countries apart from the Nordic countries [EFSA/ECDC 2019. EFSA journal 12(2):5598; EFSA/ECDC 2019. EFSA Journal 2020:18(3):6007]. Denmark is among the countries reporting the lowest occurrence of chloramphenicol and, more importantly, ciprofloxacin resistance in indicator *E. coli* from pigs, whereas the reported occurrence of ampicillin, azithromycin, sulfonamide, trimethoprim and tetracycline resistance was comparable to the average reported in the EU Member States.

The antimicrobial resistance phenotypes detected in animal-origin indicator *E. coli* mostly relevant to human health were ciprofloxacin resistance in *E. coli* from broilers and azithromycin resistance in *E. coli* from pigs, as in 2018. A slow but increasing trend in resistance to ciprofloxacin has occurred in the *E. coli* isolates from broilers over the last ten years (16% in 2019). A single ESBL-producing broiler isolate was identified. Detection of ESBL or AmpC-producing *E. coli* in Danish production animals, using the non-selective isolation procedures only, is rare. Since 2014, the non-selective monitoring has only recovered one isolate from cattle (2015) and one from broilers (2016).

Although the molecular bases of ciprofloxacin resistance have not been investigated, the phenotype indicated chromosomal mutations (in 112 of 116 ciprofloxacin resistant broiler isolates from 2014 to 2019), consequently linking the main risk to human health to the disease-causing potential of these strains. Resistance to azithromycin in isolates from pigs decreased from 4% in 2018 to 1% in 2019. The potential human risk derived by infections with these strains and/or transfer of azithromycin resistance to pathogenic strains remains low - but will be monitored closely the following years. The reduction in use of tetracycline and colistin and the increased use of macrolides and aminoglycosides since 2016, have not caused any measurable changes in the resistance patterns in *E. coli* from pigs.

Resistance to other antimicrobials relevant for human medicine such as colistin, cefotaxime, ceftazidime, meropenem and tigecycline was in general found to be very low (<1%) or was not detected. Based on the number of samples collected, absence of resistant isolates indicates a prevalence of these resistance phenotypes of less than 2% in *E. coli* from broilers, pigs and cattle (see section 9.7). However, cefotaxime- and ceftazidime-resistant *E. coli* were detected, when using selective enrichment, which is more sensitive.

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

DANMAP 2019 monitor extended-spectrum cephalosporinase- and carbapenemase-producing *E. coli* (ESC and CPE isolates) from caeca of pigs and cattle at slaughter and from Danish and imported pork and beef at retail, in concordance with the EU regulation on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food (2013/652/EU). In addition, Danish and imported broiler meat at retail was included in DANMAP 2019 as part of the national surveillance strategy. Samples were collected randomly and cultured directly in a selective enrichment for detection of cefotaxime-resistant *E. coli* and carbapenemase-producing *E. coli* (including oxacillinase-producing OXA-48-like enzymes).

Subsequently, obtained *E. coli* isolates were phenotypically antimicrobial susceptibility tested by MIC determination against the panel of defined antimicrobials and ranges of concentrations in concordance with the EU regulation. In parallel, for most isolates, whole genome sequencing (WGS) and *in silico* bioinformatics were applied to detect the ESBL, AmpC and CPE encoding genes. MIC distributions and occurrence of resistance among ESBL/AmpC-producing *E. coli* isolates are presented in the web annex (Tables A7.3 and A7.4).

7.3.1 ESBL/AmpC- and carbapenemase-producing *E. coli* from pigs and domestically produced pork

A total of 330 samples from pigs and 317 samples from domestically produced pork resulted in 89 (27%) and 9 (3%) ESC-positive samples, respectively (Table 7.2). The number of investigated samples and level of ESC isolates were slightly (non-significantly) higher than in 2017 (Figure 7.3). No CPE isolates were recovered, suggesting, with 95% confidence, that CPE isolates may be present in less than 1% of the samples from pigs and domestically produced pork.

Table 7.2 Resistance (%) and beta-lactam resistance phenotype distributions (%) in ESBL/AmpC-producing *Escherichia coli* from broilers and broiler meat recovered by selective enrichment, Denmark DANMAP 2019

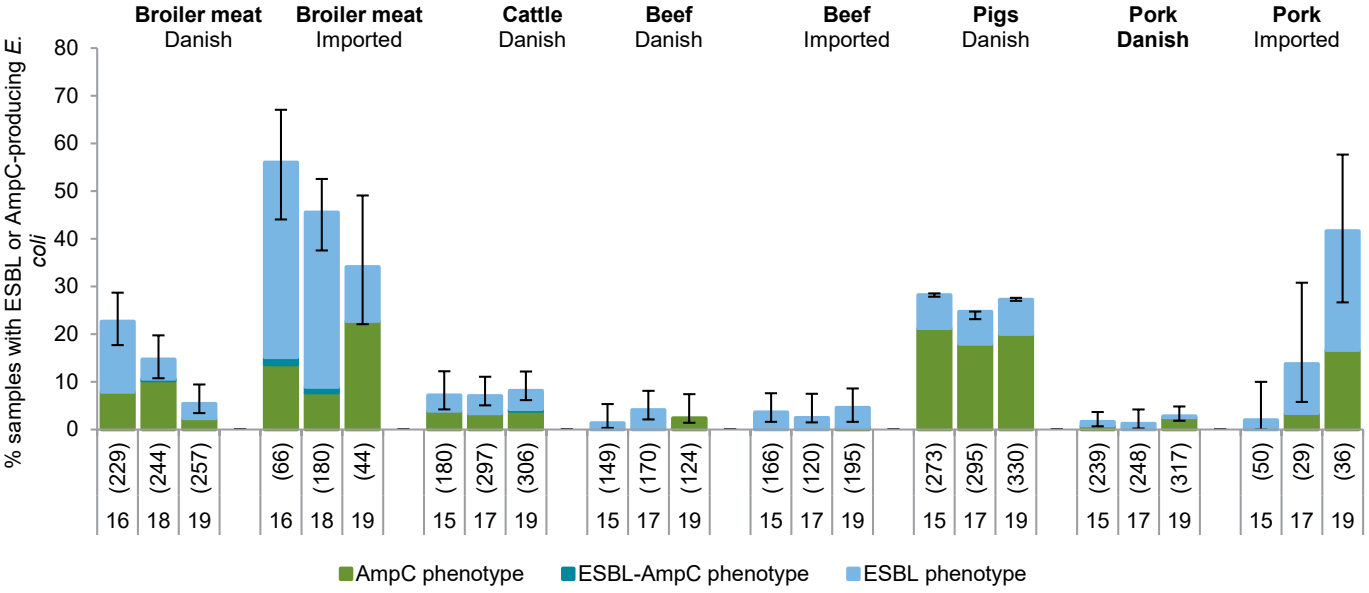
	Broiler meat		Cattle	Beef		Pigs	Pork	
Antimicrobial agent	Danish %	Import %	Danish %	Danish %	Import %	Danish %	Danish %	Import %
Ampicillin	100	100	100	100	100	100	100	100
Azithromycin	0	0	8	0	0	4	0	0
Cefepime[a]	64	53	60	0	89	36	33	87
Cefotaxime[a]	100	100	100	100	100	100	100	100
Cefotaxime/clavulansyre	43	67	48	100	11	73	89	40
Cefoxitin[a]	43	67	52	100	11	73	89	40
Ceftazidime[a]	100	93	84	100	100	100	100	100
Ceftazidime/clavulansyre	43	67	48	100	11	72	89	40
Chloramphenicol	0	0	20	0	33	9	0	0
Ciprofloxacin	57	87	16	0	56	2	11	0
Colistin	0	0	0	0	0	0	0	0
Ertapenem	0	0	0	0	0	0	0	0
Gentamicin	0	7	4	0	11	10	11	7
Imipenem	0	0	0	0	0	0	0	0
Meropenem	0	0	0	0	0	0	0	0
Nalidixic acid	57	93	4	0	44	0	0	0
Sulfonamide	29	93	56	67	33	54	44	60
Temocillin	0	0	0	0	0	0	0	0
Tetracycline	21	87	36	33	89	51	33	33
Tigecycline	0	0	0	0	0	0	0	0
Trimethoprim	7	33	36	33	56	43	44	47
CPE phenotypes	0	0	0	0	0	0	0	0
AmpC phenotypes	43	67	48	100	11	73	89	40
ESBL phenotypes	57	33	48	0	89	27	11	60
ESBL and AmpC phenotypes	0	0	4	0	0	0	0	0
Number of isolates	14	15	25	3	9	89	9	15
Number of samples	257	44	306	124	195	330	317	36

Note: Classification of CPE, ESBL and AmpC phenotypes is based on the MIC results according to the scheme provided by EFSA (see Materials and methods, section 9.7). WGS revealed ESBL genes only in the single isolates that were classified as an ESBL and AmpC phenotype according to MIC results

a) Classification of cephalosporins: 2nd generation (cefoxitin), 3rd generation (cefotaxime and ceftazidime) and 4th generation (cefepime)

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

DANMAP 2019



Note: Number of samples tested per year is presented in parentheses. Confidence intervals for total proportion of samples positive for phenotypic ESBL- and/or AmpC-producing *E. coli* - calculated as 95% binomial proportions presenting Wilson intervals. Classification of CPE, ESBL and AmpC phenotypes according to the scheme provided by EFSA (see section 9.7)

Among the 89 ESC isolates from pigs, 73% belonged to an AmpC phenotype and 27% to an ESBL phenotype, conferring 100%, 100%, 73%, and 36% resistance to cefotaxime, ceftazidime, cefoxitin and cefepime, respectively (Table 7.2).

Among the nine ESC isolates from domestically produced pork, 8 belonged to an AmpC phenotype and 1 to an ESBL phenotype, conferring 100%, 100%, 89%, and 33% resistance to cefotaxime, ceftazidime, cefoxitin, and cefepime, respectively (Table 7.2).

All, except three, of the 98 ESC isolates from Danish pigs and pork were whole genome sequenced (WGS). Among the AmpC phenotypes, most conferred resistance due to the presence of upregulated AmpC promotor C-42T mutations. One isolate had a T-32A mutation. Two additional isolates of the AmpC phenotype harboured CMY-2 encoding genes. Mainly MLSTs believed to be commensals were observed associated with the AmpC phenotype. Thus, 17, 8, 6, 7 and 5 AmpC-producing *E. coli* out of the 67 exhibiting the C-42T mutation were attributed to ST88, ST23, ST75, ST453, and ST101, respectively. ST23 is known to be observed globally and with a potential zoonotic link [Maluta et al. 2014. PLoS One 12:9(8)]. Similarly, ST48 and ST88 have previously been observed attributed to clinical cases of human and animal origin and as in isolates from domestically produced pork in Denmark, often associated to ESBL-producing *E. coli* [Sallem et al. 2014. Microb Drug Resist 20:495]. The MLST and ESBL and AmpC enzymes combinations are listed in web annex Table A7.6. The isolates which harboured the CMY-2 genotype were attributed to ST88 and ST58.

The 25 ESBL-producing *E. coli* from Danish pigs and pork were attributed to five different enzymes dominated by CTX-M-1 accounting for 20 isolates of which one in addition to CTX-M-1 also harboured CTX-M-175 gene. Similarly to the isolates of an AmpC genotype, also the enzymes of the ESBL-producing *E. coli* were attributed to a long range of MLSTs represented by only one isolate. The MLST mostly represented, but only by three isolates harbouring the CTX-M-1 gene, were ST88 (Table 7.3).

Genotypes (enzymes) of isolates from pigs were not determined in 2017, thus, a comparison to previous years was not possible. The three isolates recovered from domestically produced pork from 2017 all harboured the CTX-M-1 gene.

The ESC isolates exhibited in general a moderate level of antimicrobial resistance with no resistance to colistin, nalidixic acid, tigecycline and temocillin (Table 7.2). Resistance to fluoroquinolones was low, with only three isolates being resistant to ciprofloxacin, all harbouring *qnrS1* genes. Different combinations of additional multidrug resistance including penta-resistance in addition to ESBL- and AmpC-producing *E. coli* was observed in most isolates primarily to ampicillin, chloramphenicol, tetracycline, sulfonamides, and trimethoprim (see web annex Table A7.5).

Interestingly, resistance to gentamicin and azithromycin was observed in a few ESC isolates from pigs (4% azithromycin and 10% gentamicin resistance) and from domestically produced pork (11% gentamicin resistance).

Table 7.3 Number of ESBL and AmpC enzymes detected in *E. coli* isolates from animals and meat recovered by selective enrichment, Denmark DANMAP 2019

	Broiler meat		Cattle	Beef		Pigs	Pork	
Enzymes	Danish	Import	Danish	Danish	Import	Danish	Danish	Import
CMY-2	1					1	1	
CTX-M-1	4		3		3	19	1	7
CTX-M-14	1		6			3		1
CTX-M-15		2	3		2			
CTX-M-175						1		
CTX-M-2			1					
CTX-M-32		1				1		
CTX-M-55						1		
CTX-M-65					2			
SHV-12		1						1
Chromosomal AmpC	5	5	12	3	1	63	5	5
Not available	3	6			1	2	2	2
Number (%) of positive samples	14 (5%)	15 (34%)	25 (8%)	3 (2%)	9 (5%)	90 (27%)	9 (3%)	15 (42%)
Number of tested samples	257	44	306	124	195	330	317	36

Note: Isolates recovered by the selective enrichment methods described in the EURL-AR laboratory protocol (February 2018) and ESBL/AmpC enzymes are determined by WGS. Two isolates with CTX-M-1 contained an additional ESBL gene: CTX-M-175 from pigs and SHV-12 from imported pork. For 2019 data, all MLST and ESBL/AmpC enzymes combinations are listed in web annex Table A7.6

Co-resistance to sulfonamides and trimethoprim is likely attributable to the presence of class 1 integrons, which occasionally also harbour resistance genes encoding resistance to ampicillin, tetracycline and sometimes chloramphenicol. Moderate resistance to sulfonamides and trimethoprim was observed in the ESC isolates from pigs and domestically produced pork to both sulfonamides and trimethoprim as well as to ampicillin and tetracycline.

7.3.2 ESBL/AmpC- and carbapenemase-producing *E. coli* from cattle and domestically produced beef

A total of 306 samples from cattle and 124 samples from domestically produced beef resulted in 25 (8%) and 3 (2%) positive samples, respectively (Table 7.2). Among the isolates, no change was observed in the percentages of the different ESBL- and AmpC -phenotypes from 2017 to 2019 (Figure 7.3). No CPE isolates were recovered, suggesting, with 95% confidence, that CPE isolates may be present in less than 1% of the samples from cattle and in less than 2% of the samples from domestically produced beef.

Among the 25 ESC isolates from cattle, half belonged to an ESBL phenotype and the other half to an AmpC phenotype. The MIC results indicated one isolate of a mixed ESBL-AmpC phenotype, however, WGS analysis only revealed an ESBL gene. In contrast, the three beef isolates represented were all of the AmpC -phenotype. No statistically significant changes were observed between 2017 and 2019 (Table 7.2 and Figure 7.3). The beef isolates conferred 100% resistance to cefotaxime, ceftazidime, and cefoxitin, respectively, with no resistance to cefepime. This differs from the ESBL- and AmpC -producing *E. coli* isolates from cattle, which exhibited 100%, 84%, 52%, and 60% resistance to cefotaxime, ceftazidime, cefoxitin and cefepime, respectively (Table 7.2).

All of the three isolates from Danish beef contained the upregulated chromosomal AmpC mutations C-42T (n = 2) and T-32A (n = 1). The C-42T mutation was also observed in 12 among the 25 isolates from cattle. The remaining isolates from cattle contained the enzyme-encoding genes CTX-M-14 (n = 6), CTX-M-1 (n = 3), CTX-M-15 (n = 3) and CTX-M-2 (n = 1). Allmost all of the 28 isolates were solely attributable to single and different MLSTs. The exceptions were four and two cattle isolates containing either an upregulated chromosomal AmpC mutation, C-42T belonging to ST56 or harbouring the CTX-M-14 attributed to ST43, respectively (see web annex Table A7.6).

The cattle and beef ESC isolates exhibited in general a moderate level of antimicrobial resistance with no resistance to colistin, tigecycline, and temocillin. Moreover, no resistance among the three isolates of domestically produced beef was observed to also azithromycin, chloramphenicol, ciprofloxacin, gentamicin, and nalidixic acid. The 25 ESBL- and AmpC-producing *E. coli* isolates from cattle exhibited similar low to moderate levels of resistance to azithromycin (8%), chloramphenicol (20%), ciprofloxacin (16%), gentamicin (4%), and nalidixic acid (4%). Three of the isolates from cattle harboured *qnrS1* genes and one isolate mutations in *gyrA*.

Five ESC isolates from cattle exhibited a penta-resistance profile besides being ESBL/AmpC -producers. The resistance profile included resistance to ampicillin, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, sulfonamides, tetracycline, and trimethoprim.

7.3.3 ESBL/AmpC- and carbapenemase-producing *E. coli* from imported pork and imported beef

A total of 36 and 195 samples from imported pork and imported beef resulted in 15 (42%) and nine (5%) ESC positive samples, respectively (Table 7.2). As in previous years, the packages of meat were selected without stratification on the country of origin. Samples were also specifically examined for the presence of CPE of which none were recovered.

A statistically significant change was observed in the occurrence of ESC isolates from 2015 (2%) to 2019 (42%) among samples from imported pork (Figure 7.3). The level in imported beef was the same as in previous years. All of the 15 pork isolates and 9 beef isolates conferred resistance to cefotaxime and ceftazidime, whereas 11% and 89% of imported beef isolates and 40% and 87% of imported pork isolates were resistant to cefoxitin and cefepime, respectively (Table 7.2).

Among the 15 ESC isolates from imported pork, nine exhibited an ESBL phenotype and six an AmpC phenotype. Two isolates were not viable for WGS and the enzymes were only detected in 13 isolates from imported pork. These harboured CTX-M-1 (n = 7), CTX-M-14 (n = 1), SHV12 (n = 1) and five upregulation of chromosomal *ampC* (mutation C- 42T) (Table 7.3). A number of MLSTs were observed dominated by ST58 and ST88 associated to the CTX-M-1, CTX-M-14, SHV- 12, and upregulation of chromosomal AmpC.

One of the nine ESC isolates from imported beef, was not available for WGS. Among the remaining eight, seven displayed an ESBL phenotype and one an AmpC phenotype. Only CTX-M enzymes were observed: CTX-M-1 (n = 3, in ST117, ST446, and ST1080), CTX-M-15 (n = 2, in ST4981), and CTX-M-65 (n = 2, in ST683), reported and one isolate of an upregulated AmpC promotor, C-42T (ST88). Of the MLSTs observed in 2019, only ST446 was also observed in 2017 among isolates from imported beef.

The ESC isolates from imported beef exhibited a moderate level of antimicrobial resistance besides being ESBL producing compared to ESBL/AmpC-producing *E. coli* isolates from imported pork, which exhibited more susceptibility to many of the antimicrobials tested. No resistance to azithromycin, colistin, temocillin and tigecycline was observed for both types of imported meat with the additional absence of resistance reported for imported pork to chloramphenicol, ciprofloxacin, and nalidixic acid (Table 7.2). Two isolates from imported beef harboured *qnrS1* genes.

7.3.4 ESBL-, AmpC- and carbapenemase-producing *E. coli* from domestically produced broiler meat

A total of 257 samples from retail of domestically produced broiler meat resulted in 14 (5%) ESC positive samples, (Figure 7.3). The prevalence of ESC positive samples in 2019 was significantly lower than in 2016 (15%) and in 2018 (23%). The reason for the general reduction in the prevalence of ESC phenotypes was due to a decline of the ESBL phenotype between 2016 and 2018 from 15% to 4% combined with a decline of the AmpC phenotype between 2018 and 2019 from 10% to 2% (Figure 7.3).

No CPE isolates were recovered, suggesting that we can be 95% certain that CPE isolates are only present in 1% or less of packages of domestically produced broiler meat (see section 9.7).

Overall, the 14 ESC isolates exhibited 100%, 100%, 43%, and 64% resistance to cefotaxime, ceftazidime, cefoxitin and cefepime, respectively (Table 7.2).

A total of 11 of the ESC isolates were whole genome sequenced. The MLST and ESBL and AmpC enzymes combinations are listed in web annex Table A7.6. Among the AmpC phenotypes, five conferred resistance due to the presence of an upregulated AmpC promotor, C-42T, of which four were attributable to ST4663 (Table 7.3) whereas only one isolate harboured the CMY-2 encoding gene. The previously reported introduction of CMY-2 encoded ST2040 *E. coli* to domestically produced broiler meat seemed to disappear in 2019 compared to 2018, indicating a highly changeable population of *E. coli* present in broiler meat of Danish origin. The most common ESBL gene among the five genomes from the broiler meat was as in previous years CTX-M-1 and attributed to three MLSTs of which ST1640 previously has been reported in 2018. (see web annex Table A7.6).

The ESC isolates from Danish broiler meat exhibited varying levels of resistance to other antimicrobials. No resistance to azithromycin, chloramphenicol, colistin, gentamicin, tigecycline, and temocillin, was detected. (Table 7.2). Resistance to quinolones was still moderate but increasing, compared to previous years from 22% in 2018 to 57% in 2019. Concordance between resistance to ciprofloxacin and nalidixic acid was 100%, and chromosomal mutations in the topoisomerases genes *gyrA* were detected in one isolate.

Different combinations of multidrug-resistance were observed among the ESBL and AmpC-producing *E. coli* isolates (web annex Table A7.5). The multidrug-resistance was primarily including ampicillin (100%), ciprofloxacin, nalidixic acid, tetracycline (21%), sulfonamides (29%), and trimethoprim (7%) in addition to the cephalosporins (Table 7.2).

7.3.5 ESBL-, AmpC- and carbapenemase-producing *E. coli* from imported broiler meat

A total of 44 samples from imported broiler meat resulted in 15 (34%) ESC positive samples (Table 7.2), which was a non-significantly decrease from 2018 (46%, Figure 7.3). In contrast to the isolates from Danish broiler meat, the prevalence of ESBL and AmpC phenotypes were higher, with the AmpC phenotype being more common in imported broiler meat than in Danish meat. No CPE isolates were recovered in any of the imported broiler meat samples.

The ESC isolates exhibited 100%, 93%, 67%, and 53% resistance to cefotaxime, ceftazidime, cefoxitin, and cefepime, respectively (Table 7.2).

Only 9 of the 15 ESC isolates were available for WGS. Of those, five isolates exhibited an AmpC phenotype due to the presence of an upregulated AmpC promotor, C-42T (Table 7.3). All the AmpC-producing *E. coli* isolates originated from French products and belonged to a single MLST, ST4710, which was different from those observed in Danish broiler meat.

Interestingly, the CTX-M-1-encoding gene observed as the most commonly found ESBL phenotype in 2018 accounting for 80% of all seemed to have disappeared from the imported broiler meat. However, this could have been due to a small sample size and different producers/countries sampled in the two years. Thus, the four ESBL-producing *E. coli* isolates harboured the SHV-12, CTX-M-15, and CTX-M-32-encoding genes, respectively with three CTX-M isolates belonging to ST10 (see web annex Table A7.6). ST10 has previously been reported being emerging and pathogenic to poultry [Manges and Johnson 2012. CID 55:712; Pires-dos-Santos et al. 2013. Vet Microbiol 162(2-4):873].

The 15 ESC isolates exhibited the same trend as in previous years with higher levels of resistance and co-resistance than those from Danish broiler meat. See AMR profiles listed in web annex Table A7.5. No resistance to azithromycin, chloramphenicol, colistin, tigecycline and temocillin was observed and only low levels of resistance to gentamicin (7%) was detected (Table 7.2). Resistance to quinolones was very high with 87% (n = 13) and 93% (n = 14) resistance to ciprofloxacin and nalidixic acid, respectively. Chromosomal mutations in the topoisomerases genes *gyrA* were observed in 12 isolates. The multidrug-resistance primarily included ampicillin, chloramphenicol, tetracycline, sulfonamides, and trimethoprim in addition to the cephalosporins and often quinolones. Thus, co-resistance, likely attributable to the presence of class 1 integrons and AMR islands, was observed (Table 7.2).

7.3.6 Perspectives

The observed trends seem to be quite consistent with less variation across the years in the occurrence and level of phenotypic resistance with the exception of a significant reduction in the occurrence of ESBL- and AmpC- producing *E. coli* from Danish broiler meat and an increase in the imported pork. The enzymes of the ESBL-, AmpC- and carbapenemase-producing *E. coli* seem to be fairly consistent with the same enzymes being observed each year, but attributed to different MLSTs indicating extensive horizontal gene transfer. The extensive circulation among the commensal *E. coli* of AMR genes of critical importance might be the most interesting observation from 2019. The extensive horizontal gene transfer observed could potentially become a problem, if transferred to a pathogen or contaminating a food product normally not heat-treated.

When comparing the ESC isolates to the isolates from human bloodstream infections collected during 2019, no possible clonal relationships between isolates sharing the same combination of ST and ESBL-/pAmpC-genes, were identified by whole-genome-based single-nucleotide polymorphism (SNP) analysis. Horizontal gene transfer by plasmids encoding ESBL/pAmpC enzymes was not investigated.

Still no carbapenemase-producing *E. coli* were detected in a total of 1,609 samples tested. This is opposed to the increase in reports from other countries in Europe of incidences where carbapenemase-producing *E. coli* is emerging or persistent in the primary production [Bonardi et al. 2019. Ital J Food Saf 8:7956]. Recently, the first OXA-48-producing *E. coli* was isolated from a fattening pig in Germany and since carbapenems are not licensed for clinical use in veterinary medicine in Europe, the increase in carbapenemase-producing *E. coli* in production animals and retail meat could indicate spill-over from humans [Irrgang et al. 2020. Microorganisms 8:855].

7.4 *Enterococcus*

The *Enterococcus faecalis* and *Enterococcus faecium* isolates collected in 2019 originated from randomly collected caecal samples from healthy fattening pigs at slaughter. *E. faecalis* isolates from pigs were previously collected in 2017, whereas *E. faecium* isolates have not been collected since 2012. Since 2014, sampling and testing of *Enterococcus*, have been performed according to the EU harmonised monitoring of antimicrobial resistance [Decision 2013/652/EU] and the antimicrobial test panel recommended by EFSA was applied for MIC testing. MIC distributions and occurrence of resistance among *E. faecalis* and *E. faecium* isolates from pigs are presented in the web annex (Table A7.7). *E. faecalis* is considered intrinsically (i.e. naturally) resistant to streptogramin A and B (quinupristin/dalfopristin), and interpretation of the MIC testing for this antimicrobial agent was not done in DANMAP.

7.4.1 Enterococci from pigs

From 799 representative pig caeca collected at Danish slaughterhouses, 195 *Enterococcus* isolates were obtained and 100 *E. faecium* and 91 *E. faecalis* isolates were tested for antimicrobial resistance (Table 7.4). In total, 40 of the 100 *E. faecium* isolates (40%) showed no resistance to any of the antimicrobials in the test panel (Table 7.4).

In 2019, no linezolid, teicoplanin, tigecycline or vancomycin resistance was detected in any *Enterococcus* isolates from pigs. Among the *E. faecalis* isolates a high proportion of tetracycline (91%) and erythromycin (63%) resistance was observed whereas a more moderate proportion of chloramphenicol (33%) resistance was detected. The prevalence of ciprofloxacin (2%) and daptomycin (3%) resistance was very low in the *E. faecalis* isolates (Table 7.4).

Table 7.4 Resistance (%) in *Enterococcus* isolates from pigs, Denmark DANMAP 2019

Antimicrobial agent	*Enterococcus faecalis* %	*Enterococcus faecium* %
Ampicillin	0	12
Chloramphenicol	33	2
Ciprofloxacin	2	5
Daptomycin	3	0
Erythromycin	63	20
Gentamicin	11	1
Linezolid	0	0
Quinopristin/Dalfopristin	-	6
Teicoplanin	0	0
Tetracycline	91	54
Tigecycline	0	0
Vancomycin	0	0
Number of isolates	91	100

Note: *E. faecalis* are assumed intrinsically resistant to streptogramins and for *E. faecium* a cutoff of >4 was applied for quinopristin/dalfopristin (tradename Synercid) according to investigations presented in DANMAP 2006

The three daptomycin resistant isolates all had a MIC value of 8 mg/L, a level very close to the cutoff of >4 mg/L. The genetic background for the phenotypic daptomycin resistance was not determined.

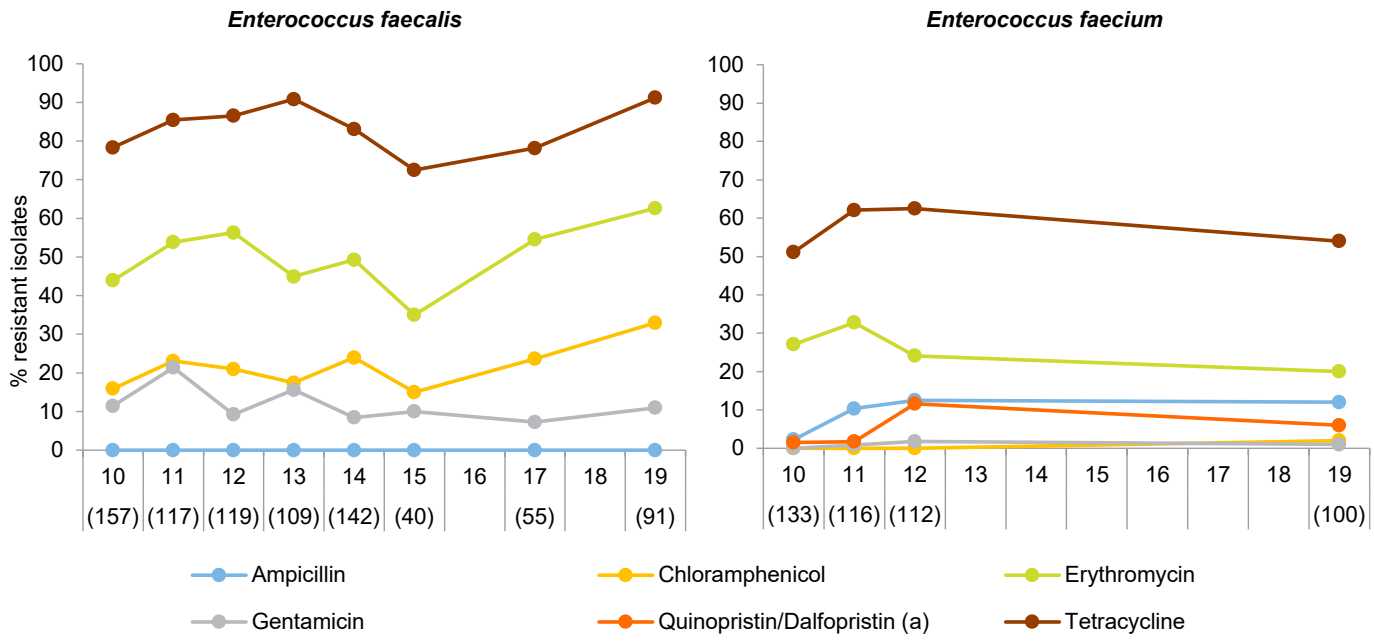
Compared to 2017, a moderate increase in chloramphenicol (9%), erythromycin (8%) and tetracycline (13%) resistance was observed whereas a very low increase in ciprofloxacin (2%), daptomycin (3%) and gentamicin (4%) resistance was detected among the *E. faecalis* isolates. In the *E. faecium* isolates, a moderate proportion of tetracycline (54%) and erythromycin (20%) resistance was found whereas low to very low proportions of ampicillin (12%), quinupristin/dalfopristin (6%), ciprofloxacin (5%), chloramphenicol (2%) and gentamicin (1%) was observed.

During the last 10-year period a statistically significant increase in chloramphenicol and erythromycin resistance have been observed in the *E. faecalis* isolates, and no ampicillin resistance has been found in this group of isolates during the last 10 years (Figure 7.4). Resistance levels in *E. faecium* were comparable to the observations from 2010-2012 (Figure 7.4).

Among the *E. faecalis* isolates, 10 resistance profiles were observed (see web annex Table A7.8). The most common profiles included resistance to i) tetracycline, ii) tetracycline, chloramphenicol and erythromycin and iii) tetracycline and erythromycin, and were observed among 29%, 25% and 21% of all *E. faecalis* isolates (n = 91), respectively.

Among the *E. faecium* isolates that showed resistance to one or more antimicrobials (n = 60), 11 resistance profiles were observed including isolates resistant to quinupristin/dalfopristin only and 15% of these isolates were resistant to three antimicrobials or more (web annex Table A7.8). The most common profiles included resistance to i) tetracycline, ii) tetracycline and erythromycin and iii) tetracycline and ampicillin, and were observed among 47%, 18% and 8% of the resistant *E. faecium* isolates, respectively.

Figure 7.4 Resistance (%) among *Enterococcus* isolates from pigs, Denmark DANMAP 2019



Note: Number of isolates included each year is presented in the parenthesis
a) *E. faecalis* are assumed intrinsically resistant to streptogramins and for *E. faecium* a cutoff of >4 was applied for quinopristin/dalfopristin (tradename Synercid) according to investigations presented in DANMAP 2006

7.4.2 Perspectives

Enterococci are commensal bacteria in the intestine in both animals and humans, however, both *E. faecalis* and *E. faecium* can cause human disease. DANMAP 2019 showed that *E. faecium* or *E. faecalis* isolates from pigs exhibited no resistance to linezolid, teicoplanin, tigecycline and vancomycin (Table 7.4). These antimicrobials are critically important to human medicine and are considered last resort compounds to treat severe infections caused by Gram positive bacteria. During the last decade, an increase in the occurrence of vancomycin-resistant *E. faecium* causing human infections, has been reported in Denmark and very few vancomycin-resistant human *E. faecalis* isolates have also been detected [DANMAP 2019. Figure 8.11].

When comparing the resistance profiles for the human *E. faecium* isolates to the resistance profiles for the *E. faecium* pig isolates from 2019, some differences can be observed. In contrast to the human isolates, the pig isolates showed no linezolid, teicoplanin or vancomycin resistance and had a much lower proportion of ampicillin resistance (12%).

Comparing resistance profiles for the *E. faecalis* isolates a few more similarities can be found. Human isolates exhibited a low proportion of linezolid resistance in contrast to no linezolid resistance among pig isolates. None of the isolates showed teicoplanin, tigecycline or vancomycin resistance. During 2019, SSI reported that vancomycin resistant *E. faecium* isolates from human clinical samples primarily belonged to three types; ST1421-CT1134 *vanA E. faecium*, ST203-CT859 *vanA E. faecium* and ST117-CT36/CT991 *vanB E. faecium* (see section 8.3).

A zoonotic link has not been reported for the VRE from human clinical samples, but studies of VRE from food and fecal samples from animals using selective VRE media are needed in order to reduce the uncertainty of such link.

Rene S. Hendriksen, Troels Ronco and Helle Korsgaard
For further information: Helle Korsgaard, hkor@food.dtu.dk