

## Prevalence of extended-spectrum cephalosporinase (ESC)-producing *Escherichia coli* in Danish slaughter pigs and retail meat identified by selective enrichment and association with cephalosporin usage

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Received 30 August 2011; returned 5 October 2011; revised 31 October 2011; accepted 7 November 2011

**Objectives:** To investigate the prevalence of extended-spectrum cephalosporinase (ESC)-producing *Escherichia coli* in pigs at slaughter and retail meat, and possible associations with the consumption of third- and fourth-generation cephalosporins.

**Methods:** During 2009, faecal samples from Danish pigs ( $n=786$ ) were collected at slaughter, and 866 meat samples [Danish: pork (153), broiler meat (121) and beef (142); and imported: pork (173), broiler meat (193) and beef (84)] were randomly collected in retail stores and outlets. *E. coli* was isolated after enrichment in MacConkey broth with ceftriaxone (1 mg/L). ESC genotypes were detected using PCR, microtube array and sequencing. The MIC of cefotaxime was determined for 150 *E. coli* from the pigs and 606 *E. coli* from meat isolated without selective enrichment.

**Results:** Eleven percent (86/786) of slaughter pigs contained ESC *E. coli* and a significantly higher prevalence was observed among pigs originating from farms with registered cephalosporin consumption in slaughter pigs ( $P=0.034$ ). Among ESC *E. coli* from pigs, 66% contained  $bla_{CTX-M-1}$ . From meat, a high prevalence of ESC *E. coli* was found in imported broiler meat (36%) compared with 0.7%–3.3% in other meat types. ESC *E. coli* from imported broiler meat ( $n=69$ ) contained  $bla_{CMY-2}$  (48%),  $bla_{CTX-M-1}$  (25%) and  $bla_{SHV-12}$  (16%). Without selective enrichment, no ESC *E. coli* from pigs and only 4.1% from imported broiler meat were found.

**Conclusions:** The usage of cephalosporins for slaughter pigs may increase the prevalence of ESC *E. coli* in slaughter pigs. Meat may be a source of ESCs in humans, especially imported broiler meat. Selective enrichment should be considered as a supplementary surveillance method.

**Keywords:** meat, swine, ESBLs, CTX-M, CMY-2

### Introduction

Extended-spectrum cephalosporinase (ESC)-producing bacteria are one of the fastest emerging resistance problems worldwide. Johnson *et al.*<sup>1</sup> found that retail foods may be an important vehicle for the community-wide dissemination of antimicrobial-resistant *Escherichia coli* and extraintestinal pathogenic *E. coli* ('ExPEC'), which may represent a newly recognized group of medically significant foodborne pathogens. Also, studies of *E. coli* from meat and urinary tract infections in Denmark suggest that *E. coli* strains from meat and production animals pose a zoonotic risk.<sup>2,3</sup> A study of healthy recruits in Denmark found extended-

spectrum  $\beta$ -lactamase (ESBL)-producing *E. coli* in faecal samples, indicating a human faecal reservoir in the community.<sup>4</sup>

A number of studies in different countries have suggested meat as a source of ESBL-producing bacteria.<sup>5–9</sup> One study found a strong correlation between ceftiofur-resistant *Salmonella enterica* serovar Heidelberg isolated from retail chicken and the incidence of ceftiofur-resistant *Salmonella* Heidelberg infections in humans across Canada. Moreover, changes in ceftiofur resistance in chicken *Salmonella* Heidelberg and *E. coli* isolates appeared related to changing levels of ceftiofur use in hatcheries during the study period.<sup>10</sup> These events provide evidence that ceftiofur use in chickens results in

extended-spectrum cephalosporin resistance in bacteria from chicken and humans.<sup>10</sup>

The use of third- and fourth-generation cephalosporins in food animal production could very well be an important reason for the occurrence of ESBL-producing bacteria among food-producing animals and in meat. In Denmark, the consumption of third- and fourth-generation cephalosporins in pig production has increased rapidly from 2001, reaching ~1‰ of the total consumption of 97.2 kg of active compound in 2008; despite this relatively low consumption, the use has been widespread in breeding herds, especially in piglets,<sup>11</sup> and may select for ESBL-producing bacteria. Since July 2010, the use of cephalosporins in Danish pig production has been close to zero due to voluntary discontinuation of their use.<sup>12</sup> Moreover, imported meat may be a source of ESBL-producing bacteria.

Until August 2003, ESBL-producing *E. coli* and *Salmonella* were not isolated from production animals or food products in Denmark.<sup>13</sup> Initial cases were all associated with imported food products or imported animals.<sup>14,15</sup> In 2005, the first two ESBL-producing *E. coli* from domestically bred pigs and cattle were reported,<sup>5</sup> and the first ESBL-producing *Salmonella* in a Danish pig herd was found in 2006.<sup>16</sup>

ESBLs in the present study are defined as the clinically important acquired  $\beta$ -lactamases with activity against extended-spectrum cephalosporins, including the classical ESBLs (CTX-M, SHV and TEM; ESBL<sub>A</sub>) and the plasmid-mediated AmpC and OXA ESBLs, classified as miscellaneous ESBLs (ESBL<sub>M</sub>).<sup>17</sup> The ESCs include ESBLs and up-regulated chromosomal *ampC*.

The aim of this study was to investigate the prevalence of ESBLs in Danish pigs at slaughter and in Danish and imported retail meat. Further, we aimed to reveal any association between the presence of ESC *E. coli* and the consumption of third- or fourth-generation cephalosporins. The study also compares a selective enrichment method to the findings of cefotaxime resistance in indicator *E. coli*.

## Materials and methods

### Sampling procedure

Pigs were sampled at slaughter from 11 slaughter plants in Denmark. These slaughter plants represented 94% of the total number of pigs slaughtered in Denmark. The sampling was done as a weighted sampling, meaning that the number of samples taken at a particular slaughter plant was proportional to the number of pigs slaughtered at each plant per year. This sampling procedure resulted in 2–17 faecal samples collected at each plant each month as caecal samples after evisceration. The sampling was done in the last week of every month from February through November 2009. Only one sample was collected from each Danish Central Husbandry Register (CHR) number each month. The CHR contains data on the farm, such as the owner, address, animal species and number of animals in each age group (sows/piglets, weaning pigs and finishers). The sampling was done by meat inspection staff or company personnel. The samples were sent in sterile plastic containers to the Danish Veterinary and Food Administration (DVFA) regional laboratories for analysis.

The meat samples were collected randomly in retail stores and outlets in all regions of Denmark by the Regional Veterinary and Food Agency Authorities. Only samples of raw meat (frozen or refrigerated) were included. Sample information included: origin of meat, meat type, sampling site, sampling day, production date and expiry date. The food samples were collected following the DVFA guidelines for the microbial

examination of foods.<sup>18</sup> The samples were analysed on the day of sampling at the DVFA regional laboratories.

### Isolation of ESC *E. coli*

Presumptive ESC-producing *E. coli* were isolated by adding 1 g of faeces to 10 mL of MacConkey broth (Oxoid CM5a) supplemented with 1 mg/L ceftriaxone (Sigma C5793-1G) and incubating for 16–18 h at 44°C. A 10  $\mu$ L aliquot was streaked on MacConkey agar supplemented with 1 mg/L ceftriaxone, incubated overnight at 44°C and a maximum of three colonies were subcultured. The same procedure was used for meat samples, except that 5 g of meat was used. *E. coli* were identified on CHROM Orientation agar (Becton Dickinson a/s).

### Indicator *E. coli*

All meat samples ( $n=866$ ) and 284 of the pig faecal samples were used for isolation of *E. coli* without selective enrichment as part of the Danish Integrated Antimicrobial Resistance Monitoring and Research Program.<sup>11</sup> The isolates from pigs were identified as *E. coli* by use of Drigalski agar followed by CHROM Orientation agar. From meat, isolation was performed by adding 5 g of the sample to 45 mL of MacConkey or laurylsulphate broth, incubating overnight at 44°C and subsequently streaking onto violet red bile agar and incubating for 24 h at 44°C. Presumptive *E. coli* were further identified by CHROM Orientation agar.<sup>11</sup> All 606 *E. coli* isolates obtained from the meat samples and 150 of the 278 isolates obtained from pig faecal samples (a representative randomly chosen subset) were tested for susceptibility to cefotaxime by use of Sensititre (Trek Diagnostic Systems Ltd, UK), following CLSI guidelines.<sup>19</sup> Cefotaxime-resistant *E. coli* were determined by use of the EUCAST epidemiological cut-off value ( $>0.25$  mg/L). The *E. coli* strain ATCC 25922 was used as a quality control.

### Detection of ESC genes

The ESC genotype was determined by use of PCR, sequencing and microtube DNA array. The isolates were screened for the ESC genotypes most commonly found in the reservoirs (pig or meat) and based on ESBL phenotype for: ESBL<sub>M</sub> (cefoxitin<sup>r</sup> and cefepime<sup>s</sup>), ESBL<sub>A</sub> (cefoxitin<sup>s</sup> and reduced in susceptibility to cefepime; resistance to ceftazidime and/or cefotaxime and synergy to clavulanic acid) or unclear interpretation. Disc diffusion was performed according to CLSI guidelines using the following discs (Oxoid, UK): cefoxitin, 30  $\mu$ g; cefepime, 30  $\mu$ g; ceftazidime, 30  $\mu$ g; cefotaxime, 30  $\mu$ g; cefotaxime+clavulanic acid, 30+10  $\mu$ g; and ceftazidime+clavulanic acid, 30+10  $\mu$ g.<sup>19</sup> For quality control, *E. coli* ATCC 25922 was used. The results were interpreted by use of the following interpretive criteria: the isolates were determined as resistant to cefoxitin, cefepime, ceftazidime or cefotaxime if zone diameters were  $\leq 14$  mm; and clavulanic acid synergy with ceftazidime or cefotaxime was interpreted as an increase in zone diameter  $\geq 5$  mm between ceftazidime and ceftazidime+clavulanic acid, and cefotaxime and cefotaxime+clavulanic acid, respectively.

ESBL<sub>M</sub> meat isolates were first screened for *bla*<sub>CMY-2</sub> by PCR and sequencing; if negative, the isolates were further screened for up-regulated *ampC* by PCR and sequencing. The rest of the ESBL<sub>M</sub>s from meat were screened by use of a microtube DNA array system (Clondiag, Germany) and Identibac Amr-ve array tubes (New Haw, Addlestone, Surrey, UK);<sup>20</sup> based on the array result, PCR and sequencing were done. For ESBL<sub>M</sub> from pigs the same screening strategy was used, except up-regulated *ampC* was determined before *bla*<sub>CMY-2</sub>.

ESBL<sub>A</sub> isolates from both pigs and meat were screened for *bla*<sub>CTX-M</sub> by PCR and further sequenced for determination of the *bla*<sub>CTX-M</sub> type. If the isolates were negative for *bla*<sub>CTX-M</sub>, they were screened for *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> by PCR followed by sequencing. For isolates negative for these two

PCRs, the microtube DNA array was used. Based on the array result, PCR and sequencing were done in order to determine the exact gene responsible for the reduced susceptibility towards cephalosporins. Isolates with an unclear phenotypical interpretation were screened for up-regulation of the chromosomal *ampC* promoter and for the presence of *bla*<sub>CTX-M</sub> genes. For isolates negative for up-regulated *ampC* or *bla*<sub>CTX-M</sub> genes, the microtube DNA array system was used followed by PCR and sequencing.

The following primers were used for PCR, as previously described: *bla*<sub>CMY-2</sub> (CMY-2start: 5'-ATG ATG AAA AAA TCG TTA TGC TGC-3', CMYgroup2-R: 5'-GCT TTT CAA GAA TGC GCC AGG-3'); *bla*<sub>CTX-M</sub> (CTX-M-U1: 5'-ATG TGC AGY ACC AGT AAR GTK ATG GC-3', CTX-M-U2new: 5'-GGG TRA ART ARG TSA CCA GAA YSA GCG G-3'); *bla*<sub>SHV</sub> (SHV-OS5: 5'-TTA TCT CCC TGT TAG CCA CC-3', SHV-OS6: 5'-GAT TTG CTG ATT TCG CTC GG-3'); *bla*<sub>TEM</sub> (TEMCRny: 5'-ACC AAT GCT TAA TCA GTG AG-3', TEM-frontp1: 5'-GCG GAA CCC CTA TTT G-3'); and *ampC* (*ampC*nyP1: 5'-GTT GTT TCC GGG TGA TGC-3', *ampC*-R: 5'-TGG AGC AAG AGG CGG TA-3').<sup>21-24</sup> The positive control strains were *Salmonella* Heidelberg 75-12893-1 (*bla*<sub>CMY-2</sub>), *E. coli* O:149 77-30108-11 (*bla*<sub>CTX-M</sub>), *E. coli* 76-33094-7 (*bla*<sub>TEM</sub>), *E. coli* ampC+ 16 Holland (up-regulated *ampC*) and *Salmonella* Keurmassar DAK-2 (*bla*<sub>SHV</sub>).

### Data on farms and consumption of third- and fourth-generation cephalosporins

The data were obtained from the VetStat database. In Denmark, all therapeutic drugs are prescription-only. Reporting to the VetStat database on all medicines prescribed for use in animals has been mandatory since 2001. The VetStat database contains detailed information about the source and consumption for each prescription item: date of sale, identity of prescribing veterinarian, source ID (pharmacy, feed mill or veterinarian), package identity code (Nordic item number) and amount, animal species, age group, disease category and code for farm identity (CHR number). The Nordic item number relates to all product information at the package level. The number of farms included was based on the ones active in February 2009. Two sets of data were produced: one with cephalosporin consumption for slaughter pigs (all farms) and one with consumption for any age group within the farm (integrated farms only). Farms were defined as having used cephalosporins in one of two ways: usage of third- or fourth-generation cephalosporins at least once in the 6 months prior to sampling; or usage of third- or fourth-generation cephalosporins at least once in the 12 months prior to sampling. The data sets for cephalosporin usage in slaughter pigs 6 and 12 months prior to sampling included two and three farms with usage in an unspecified age group, respectively. For animals traded during their lifespan, only consumption on the last farm (sending to slaughter) was included in the analysis. Thus, for integrated farms, consumption in all age groups (sections) present was included, while for pigs originating from farms housing only slaughter pigs, only consumption in the slaughter pigs was obtained.

Statistical significance tests of differences between proportions of ESC-producing *E. coli* (with and without third- and fourth-generation cephalosporin consumption) were calculated using StatCalc in EpiInfo™ v. 6. Statistically significant differences were calculated using a  $\chi^2$  test or Fisher's exact test (two-tailed) when the number of samples was low (<25). The estimation of exact 95% (two-sided) CIs for proportions was based on binominal probability distributions, as described by Armitage and Berry.<sup>25</sup>

## Results and discussion

### Origin of samples and correlation between phenotype and genotype

For isolation of ESC-producing *E. coli*, 786 faecal samples and 866 meat samples [Danish: pork ( $n=153$ ), broiler meat

( $n=121$ ) and beef ( $n=142$ ); and imported: pork ( $n=173$ ), broiler meat ( $n=193$ ) and beef ( $n=84$ )] were tested. Of the 786 faecal samples, 679 farms active in 2009 February could be matched by comparing CHR numbers from samples and farms. Of these, 62% only housed slaughter pigs, 16% housed weaning pigs and slaughter pigs, and 21% housed slaughter pigs, weaning pigs and sows/piglets (Table 1). The imported meat samples originated mainly from countries in the European Union (EU), with most samples originating from Germany, France (mainly broiler meat) and the Netherlands (mainly pork and beef) (71%, 8% and 8%, respectively) (Table 2).

Thirty-six and 13 *E. coli* isolated from different meat and pigs, respectively, had the ESBL<sub>M</sub> phenotype. Most of these isolates carried the *bla*<sub>CMY-2</sub> gene ( $n=35$ ) while the remaining ( $n=13$ ) had an up-regulated *ampC* promoter or an unknown mechanism ( $n=1$ ). Forty-two and 66 from meat and pigs, respectively, had the ESBL<sub>A</sub> phenotype, and these isolates had either *bla*<sub>CTX-M</sub> ( $n=90$ ), *bla*<sub>SHV-12</sub> ( $n=12$ ), *bla*<sub>TEM-20</sub> ( $n=1$ ) or unknown mechanisms ( $n=5$ ). Nine isolates had an unclear phenotypical interpretation and these isolates contained up-regulated *ampC* ( $n=1$ ), *bla*<sub>CTX-M</sub> ( $n=4$ ), *bla*<sub>TEM-20</sub> ( $n=1$ ), *bla*<sub>TEM-52</sub> ( $n=2$ ) or an unknown mechanism ( $n=1$ ). A clear correlation between the ESBL phenotype and ESBL genes was found for 153 out of 166 isolates, and for only 7 isolates could the genotype not be determined. Therefore, the information about either the ESBL<sub>M</sub> or ESBL<sub>A</sub> phenotype combined with the information about the reservoir was useful when screening for genes.

### Occurrence of ceftriaxone-resistant *E. coli* and distribution of ESC genes

Eighty-six (11%) of the faecal samples from pigs at slaughter contained ESC-producing *E. coli*. Among these 86 *E. coli* isolates from slaughter pigs, 57 (66%) contained a *bla*<sub>CTX-M-1</sub> gene while other genotypes were found less frequently [up-regulated *ampC* (15%), *bla*<sub>CTX-M-14</sub> (7%), *bla*<sub>CTX-M-15</sub> (2%), *bla*<sub>CTX-M-2</sub> (4%), *bla*<sub>SHV-12</sub> (1%), *bla*<sub>TEM-20</sub> (1%) and unknown mechanisms (4%)] (Figure 1). From Danish pork, a prevalence of 2.0% was found for ESC-producing *E. coli*, corresponding to two *bla*<sub>CTX-M-2</sub> genes and one of unknown type. This was surprising, since *bla*<sub>CTX-M-1</sub> was the most commonly found type in pigs. This may be due to (i) differences in the survival in the food production chain of the *E. coli* with *bla*<sub>CTX-M-1</sub> and *bla*<sub>CTX-M-2</sub>, respectively, or (ii) to cross-contamination during slaughter or post-slaughter. A study of Danish human *E. coli* isolates from blood infections isolated in four out of five regions of Denmark in 2009 found *bla*<sub>CTX-M-15</sub> in 83% of the isolates and *bla*<sub>CTX-M-1</sub> in 9%, whereas *bla*<sub>CTX-M-2</sub> was not found.<sup>26</sup> The *bla*<sub>CTX-M-15</sub> gene has also been commonly found in *E. coli* from human infections in other countries.<sup>27-30</sup> There was a low prevalence of *bla*<sub>CTX-M-15</sub> in pigs in our study. This could be due to cross-contamination from slaughter personnel, but since the samples were taken 'sterile' from the caecum they most likely originate from the pigs and may thereby contribute to resistance in humans.

Even though ESBL-producing *E. coli* were present in Danish pigs at slaughter, the most important meat source seemed to be imported broiler meat. Among the meat samples a high prevalence of ESC-producing *E. coli* was found among imported broiler meat (36%), while the other meat categories (of Danish or imported origin) contained 0.7%–3.3% (Table 2). The 69 ESC-

**Table 1.** Different farm types and sizes of farms included in the study

Farm types pigs originated from	No. of farms	ESC-positive farms	Cephalosporins prescribed for slaughter pigs (<12 months prior to sampling)	Cephalosporins prescribed for slaughter pigs (<6 months prior to sampling)
Slaughter pigs only, no. of pigs	<b>424</b>	11.8% (50)	2.8% (12)	2.4% (10)
<1000	152			
1000–2500	230			
>2500	42			
Slaughter pigs and weaning pigs, no. of pigs	<b>111</b>	10.0% (11)	1.8% (2)	1.8% (2)
<1000	42			
1000–2500	56			
>2500	13			
Sows, weaning pigs and slaughter pigs, no. of pigs	<b>144</b>	10.4% (15)	3.5% (5)	2.8% (4)
<250	69			
250–500	53			
>500	22			
no. of farms with weaning pigs	134			

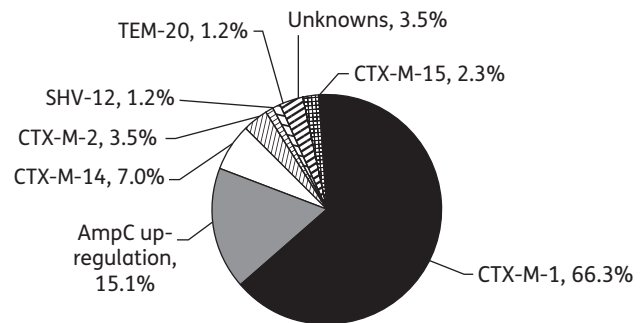
Slaughter pigs only: farms that only have slaughter pigs.  
 Slaughter pigs and weaning pigs: farms that have slaughter pigs and weaning pigs.  
 Sows, weaning pigs and slaughter pigs: integrated farms that have sows/piglets, weaning pigs and slaughter pigs.  
 Numbers in parentheses represent the number of farms.

**Table 2.** Meat samples and countries the meat originates from

Country	Pork		Beef		Broiler meat	
	n	% ESC positive	n	% ESC positive	n	% ESC positive
<b>Denmark</b>	<b>153</b>	<b>2.0</b>	<b>142</b>	<b>0.7</b>	<b>121</b>	<b>3.3</b>
Germany	142	0.7	27	0	149	34
Netherlands	16	0	17	5.9	1	100
France	1	0	0	—	35	43
Poland	1	0	16	0	0	—
Other EU countries	12	8.3	12	0	6	0
Third countries	1	0	12	0	2	100
<b>Imported total</b>	<b>173</b>	<b>1.2</b>	<b>84</b>	<b>1.2</b>	<b>193</b>	<b>36</b>

n, total number of samples.  
 Other EU countries (n < 10 for all meat types): Belgium, Ireland, Lithuania, Spain, Sweden and the UK.  
 Third countries (n < 7 for all meat types): Argentina, Australia, Brazil and the USA.

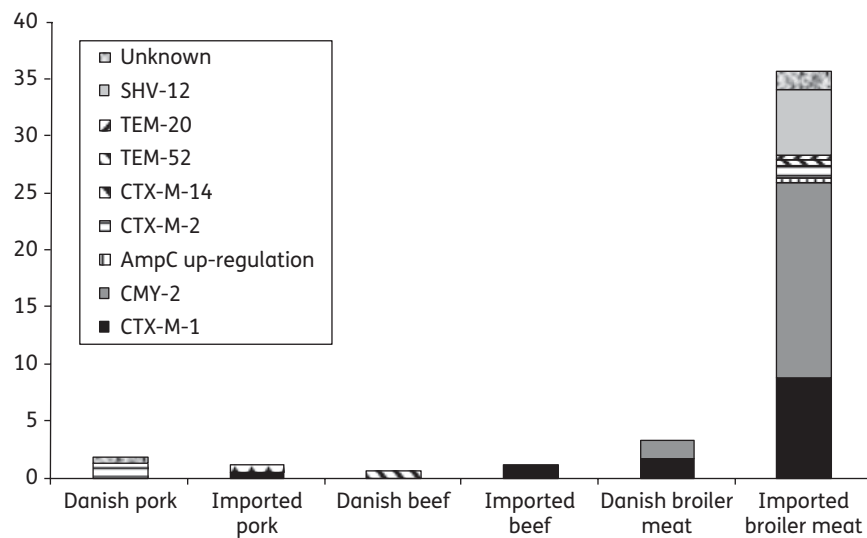
producing *E. coli* isolates from imported broiler meat contained *bla*<sub>CMY-2</sub> (48%), *bla*<sub>CTX-M-1</sub> (25%), *bla*<sub>SHV-12</sub> (16%), *bla*<sub>CTX-M-2</sub> (3%), other mechanisms (*bla*<sub>TEM-20</sub>, *bla*<sub>TEM-52</sub> and *ampC* up-regulation;



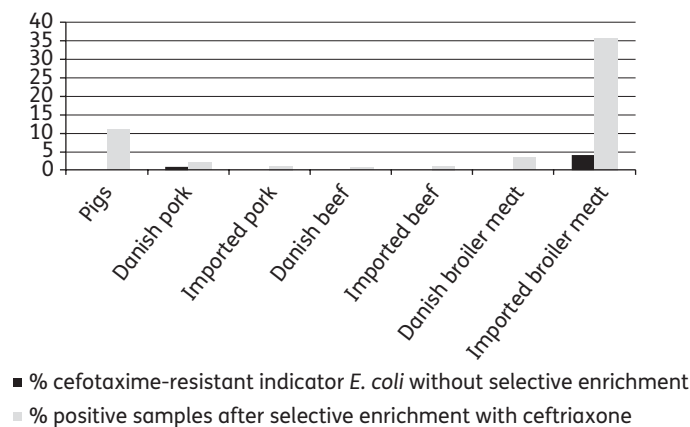
**Figure 1.** Distribution of ESC genes among ESC-producing *E. coli* from pigs at slaughter.

4%) and unknown mechanisms (4%). Among the other meat categories, *bla*<sub>CMY-2</sub>, *bla*<sub>CTX-M-1</sub>, *bla*<sub>CTX-M-2</sub>, *bla*<sub>CTX-M-14</sub>, *bla*<sub>TEM-52</sub> and up-regulated *ampC* were found (Figure 2). A recent study of ESBL in *E. coli* from patients, retail chicken and poultry in the Netherlands showed that the same strains and genes could be found in humans, retail meat and poultry, indicating transmission to humans.<sup>8</sup> Another study of ESBL-producing *E. coli* from clinical samples and retail meats in Pittsburgh, USA and Seville, Spain found *E. coli* of the same phylotypes and with the same ESBL genes present in meats, pigs and humans.<sup>31</sup> Therefore, it is likely that ESBL-producing bacteria present in animals could be the origin, at least in part, of the human cases.





**Figure 2.** Prevalence of ESC-producing *E. coli* (%) and distribution of ESC genes in different meat types of Danish and imported origin.



**Figure 3.** Prevalence of ESC-producing *E. coli* obtained after selective enrichment and ESC-producing indicator *E. coli* obtained without selective enrichment.

Most imported broiler meat samples originated from Germany ( $n=149$ ) or France ( $n=35$ ). Of these samples, 34% and 43%, respectively, contained ESC-producing *E. coli*. One hundred and three out of the 149 samples from Germany contained information about which country the animal was raised in, and all 103 of these samples originated from broilers raised in Germany. The majority of samples ( $n=140$ ) came from three slaughterhouses.  $bla_{CMY-2}$ - and  $bla_{CTX-M-1}$ -positive samples were obtained from all three slaughterhouses, whereas all four isolates with  $bla_{SHV-12}$  originated from the same slaughterhouse. The four meat samples were collected on different dates (March–June) in a retail shop and an outlet, and at least one of the samples (sampled in April) was refrigerated meat. Only seven of the samples from France contained information on which country the animal was raised in; these samples all originated from France, and four of these samples contained ESC-producing *E. coli* with  $bla_{CTX-M-1}$ ,  $bla_{SHV-12}$ ,  $bla_{CMY-2}$  and unknown type, respectively, from four different slaughterhouses. Therefore, there are several broiler production units in the EU contributing to the occurrence of ESBLs. This is contrary to an earlier study

in Denmark, which found  $bla_{CTX-M-1}$  and  $bla_{CMY-2}$  *E. coli* isolated from broilers to originate from a single slaughterhouse in Germany.<sup>6</sup> Even though only two ESBL-positive isolates originated from third countries, some of the samples without information on what country the animal was raised in may originate from these countries (Table 2). As certain genotypes were dominant in *E. coli* from certain meat sources, the genotype may be valuable for future source attribution models.

### Comparison between methods

The use of selective enrichment with ceftriaxone revealed ESC-producing *E. coli* that were not found by standard monitoring of indicator *E. coli* in pigs and in four out of six meat categories. Ceftriaxone (1 mg/L) has been found previously to be the best choice for the selective enrichment of eight cephalosporins for the detection of ESC-producing *E. coli*.<sup>32</sup> Imported broiler meat had the highest prevalence of ESC-producing *E. coli* with both selective enrichment and when using standard monitoring (Figure 3). The finding that more than one-third of the broiler meat samples

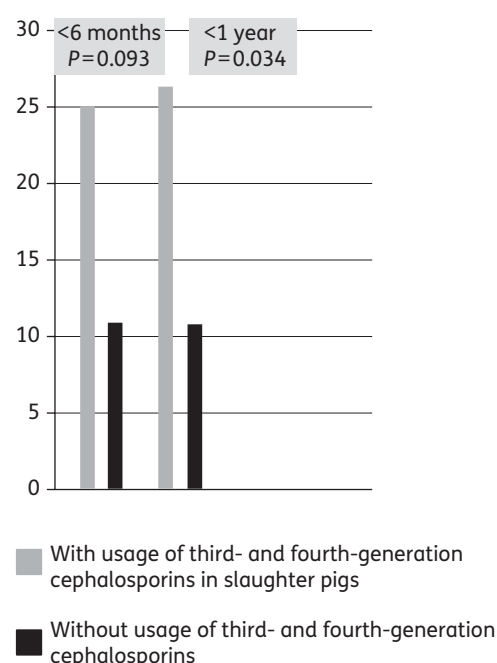
were positive was surprising, and selective enrichment seems to be a good supplement to standard monitoring and should be considered for emerging resistance types in relevant reservoirs.

### Usage of third- and fourth-generation cephalosporins in farms and occurrence of cephalosporinase-producing *E. coli*

The use of third- and fourth-generation cephalosporins is likely to select for ESC-producing *E. coli*. In Denmark, 99 kg of active compound was used in 2009 and was mainly (85%) prescribed for sows/piglets at 792 farms; of these, 392 also had third- or fourth-generation cephalosporins prescribed for another age group.<sup>11</sup> Information on the use of cephalosporins in this present study was obtained for 679 of the farms. Of these farms, 16 and 19 had used cephalosporins (third- or fourth-generation, mainly ceftiofur and cefquinome) for slaughter pigs at least once within the past 6 or 12 months, respectively, before the samples were collected. There was a statistically significant ( $P=0.034$ ) higher prevalence [26.3% (9.2%–51.2%) versus 10.8% (8.5%–13.4%)] of ESC-producing *E. coli* from farms with third- or fourth-generation cephalosporin usage for slaughter pigs at least once in the 12 months prior to sampling, whereas for farms with third- or fourth-generation cephalosporin usage for slaughter pigs at least once in the 6 months prior to sampling the higher prevalence was not significantly different ( $P=0.093$ ) [25.0% (7.3%–52.4%) versus 10.9% (8.6%–13.5%)], probably due to the small sample size (Figure 4). The farms that used third- or fourth-generation cephalosporins for slaughter pigs at least once in the 12 months prior to sampling accounted for 2.8% of the farms (Table 1). Farms with third- or fourth-generation cephalosporin consumption for slaughter pigs were not more likely to have sows/piglets or weaning pigs: 144 of the farms were fully integrated (including sows, weaning pigs and slaughter pigs) and 3.5% ( $n=5$ ) had used third- or fourth-generation cephalosporins for slaughter pigs 12 months prior to sampling. Among the 424 farms only having slaughter pigs registered, 2.8% ( $n=12$ ) had used third- or fourth-generation cephalosporins for slaughter pigs at least once in the 12 months prior to sampling. Among the 111 farms having weaning pigs and slaughter pigs registered, 1.8% ( $n=2$ ) had third- or fourth-generation cephalosporin consumption for slaughter pigs at least once in the 12 months prior to sampling (Table 1).

The influence of third- or fourth-generation cephalosporin consumption in any age group on the occurrence of ESC *E. coli* in pigs at slaughter was investigated in the integrated farms (144 farms). Thirty and 36 integrated farms had used third- or fourth-generation cephalosporins in any age group at least once in the period up to 6 or 12 months before sampling, respectively. The occurrence of ESBL-producing *E. coli* in the slaughter pigs was not significantly higher in integrated farms with consumption of third- or fourth-generation cephalosporins in the pig production in any age group when compared with farms with no usage of cephalosporins (data not shown). One explanation for this could be that ESC *E. coli* are outcompeted by other bacteria when cephalosporins are absent in weaning pigs or slaughter pigs, but this needs to be further studied, especially as other factors such as ampicillin consumption or co-selection for other antimicrobials may play a role.

In conclusion, it is recommended to supplement standard surveillance methods with methods based on selective enrichment



**Figure 4.** Correlation between consumption of third- and fourth-generation cephalosporins for slaughter pigs in the farms prior to sampling and the presence of ESC-producing *E. coli* in pigs at slaughter. <6 months, third- or fourth-generation cephalosporin consumption for slaughter pigs at least once in the 6 months prior to sampling. <1 year, third- or fourth-generation cephalosporin consumption for slaughter pigs at least once in the 12 months prior to sampling. The y-axis represents the percentage of farms positive for ESC *E. coli*.

when monitoring ESBLs. The finding of ESBL genotypes in pigs or broiler meat that can be found in humans indicates that food-producing animals may be the origin in at least part of the human cases and information on ESBL genotype may be valuable for source attribution. The consumption of third- and fourth-generation cephalosporins in slaughter pigs may select for ESC-producing *E. coli* in pig production. The finding of ESBLs in more than one-third of the imported broiler meat samples suggests imported broiler meat as a source of ESBLs in humans and the presence may be due to the consumption of cephalosporins in broiler production, but this should be further investigated.

### Acknowledgements

We would like to acknowledge Christina Aaby Svendsen and Hanne Nørgaard Nielsen for excellent technical assistance, and the regional laboratories administrated by the Danish Food Agency for collecting the samples and isolating the bacteria.

### Funding

The work was partly funded by the Danish Ministry of Food, Agriculture and Fisheries (grant no. 3304-FVFP-09-F-002-1) and the Danish Food Agency 'centrally coordinated projects'.

## Transparency declarations

None to declare.

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