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Original Article

Spread of Extended Spectrum Cephalosporinase-Producing *Escherichia coli* Clones and Plasmids from Parent Animals to Broilers and to Broiler Meat in a Production Without Use of Cephalosporins

Yvonne Agersø,[1] Jacob Dyring Jensen,[1] Henrik Hasman,[1] and Karl Pedersen[2]

Abstract

Objectives: This study investigated the occurrence of extended spectrum cephalosporinase (ESC)–producing *Escherichia coli* in a broiler production with no cephalosporin use and a low use of antimicrobials in general. Furthermore, it investigated whether the current consumption of aminopenicillins selects for ESC-producing *E. coli* and whether certain clones or plasmids spread from imported parent flocks to the meat.

Materials and Methods: ESC-producing *E. coli* was isolated using MacConkey broth with 1 mg/L of ceftriaxone. ESC genes were identified using polymerase chain reaction and sequencing. Isolates with $bla_{\text{CMY-2}}$ were subtyped by pulsed-field gel electrophoresis (PFGE), phylotyping, and antimicrobial susceptibility testing. Selected isolates were used as donors in filter-mating experiments, multilocus sequence typing (MLST), and plasmid replicons were typed. Aminopenicillin use at the farm (not flock) level was obtained from VetStat, a database for mandatory registration of veterinary prescriptions in Denmark.

Results: ESC-producing *E. coli* occurred in 93% (27/29) of broiler parent farms in 2011, 27% (53/197) of broiler flocks in 2010, and 3.3% (4/121) of Danish retail broiler meat in 2009 and 8.6% (16/187) in 2010. The ESC producing *E. coli* contained $bla_{\text{CMY-2}}$, $bla_{\text{SHV-2}}$ or $bla_{\text{CTX-M-1}}$. Isolates with $bla_{\text{CMY-2}}$ represented 35 PFGE groups. One group dominated (39 isolates) and included isolates with indistinguishable PFGE patterns from parents, broilers, and meat. Most $bla_{\text{CMY-2}}$ isolates were susceptible to non-β-lactams, and $bla_{\text{CMY-2}}$ was mostly present on horizontally transferable incI1 or incK plasmids. Phylogroup D was most common and *E. coli* MLST types previously found in humans were observed. Broiler farms with registered aminopenicillin use had significantly higher occurrence of ESC *E. coli*.

Conclusions: ESC-producing *E. coli* from flocks of imported broiler parents spread clonally and horizontally to broiler meat (including potentially human pathogenic types) even in a country with no cephalosporin use. Use of aminopenicillins may influence the persistence of ESC-producing *E. coli* in the broiler production, but other factors should be investigated.

Introduction

Extended-spectrum-β-lactamase and plasmid-mediated AmpC resistance (extended-spectrum cephalosporinases [ESC]) are some of the fastest-emerging resistance problems worldwide (EFSA BIOHAZ, 2011). Production animals and their meat products (especially broiler meat) seem to be a source for ESC-producing bacteria causing infection in humans (Dutil *et al*., 2010; Leverstein-van Hall *et al.,* 2011; Overdevest *et al.,* 2011; Kluytmans *et al*., 2013). Broiler production in Europe is characterized by having very few distributors of grandparent animals, and since ESC is widespread and often caused by the same types $bla_{\text{CMY-2}}$ or $bla_{\text{CTX-M-1}}$ present on horizontally transferable plasmids, ESC may have spread through the breeding chain from the very top of the breeding pyramid to the bottom (EFSA BIOHAZ, 2011). Moreover, the plasmids may have spread to other pathogen bacteria.

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The aim was to investigate the occurrence of ESC-producing *E. coli* in a broiler production with no use of cephalosporins and a low use of antimicrobial agents in general. Furthermore, this study investigated whether the current consumption of aminopenicillins selects for ESC-producing *E. coli* and whether certain clones or plasmids spread from imported parent flocks to retail broiler meat.

In Denmark, ESC-producing *Escherichia coli* in the Danish produced broiler meat was identified for the first time in 2009 (Agersø *et al.*, 2012). The occurrence was low (less than 10%) in 2009 and in 2010 when compared to broiler meat imported into Denmark (DANMAP 2010, 2011). In 2011, ESC-producing *E. coli* increased significantly in the Danish-produced broiler meat to the same level as in imported broiler meat (44% and 48%, respectively) (DANMAP 2011, 2012). Cephalosporins have never been approved for use in poultry in Denmark, and no use of cephalosporins for poultry has been registered for at least a decade. In general, the consumption of antimicrobials in the Danish broiler production is low compared to other production animals, as only approximately 30% of the farms have had antimicrobials prescribed (414 kg, 182 prescriptions) corresponding to only 3% of the flocks treated in 2011 (DANMAP 2010, 2011; DANMAP 2011, 2012). However, in 2011, the consumption of aminopenicillins, the major drug of choice, was two to five times higher than in previous years (DANMAP 2010, 2011, 2012). ESC-producing *E. coli* produces β-lactamases that can degrade aminopenicillins, but to what extent aminopenicillins select for ESC-producing bacteria is unknown.

Materials and Methods

Sampling procedure

Samples were collected from all conventional parent flocks hatching broiler eggs, from randomly selected broiler flocks at slaughter, and from randomly selected Danish broiler meat. Boot swab samples (sock samples) were collected from the parent flock houses by the farm owners during September 2011. Sterile gauze socks were placed on clean boots, the sample collector walked around in the parent flock, and a house (flock) was sampled once. Up to four socks from different houses within the same herd were pooled and sent to the National Food Institute for analysis. Farms with one to four houses were examined as one pooled sample consisting of one sock from each house, while farms with more than four houses were examined as two pooled samples. No farm had more than six houses. In total, 29 farms were sampled, resulting in 70 sock samples pooled into 32 samples.

The broiler flocks were sampled weekly before slaughter at the five slaughterhouses in Denmark slaughtering conventional produced broilers from May through November as part of the DANMAP program, and the Central Husbandry Register (CHR) number of the farm was registered (DANMAP 2010, 2011). Cloacal swabs from five broilers of the same flock were randomly collected by slaughterhouse personnel and pooled into one sample representing approximately 60% of all broiler farms in Denmark. No flock was sampled more than once. This procedure resulted in sampling of 87 different farms with 1–2 flocks sampled and 13 farms with 3–15 flocks sampled.

Three milliliters of 0.9% NaCl was added to each sample of pooled swabs before sending it to the regional laboratories for analysis.

The meat samples were collected randomly in retail stores and outlets in all regions of Denmark as part of the DANMAP program (Agersø *et al.*, 2012; DANMAP 2010, 2011).

Analysis of the samples

Presumptive ESC-producing *E. coli* was isolated from meat as previously described (Agersø *et al.,* 2012). From parent farms, 1 to 4 sock samples were added to 225 mL of MacConkey (Oxoid CM5a, Basingstoke, England) broth supplemented with 1 mg/L of ceftriaxone (Sigma C5793-1G, Steinheim, Germany) and incubated for 16–18 h at 44°C. Ten microliters of this enrichment broth was then streaked on a MacConkey agar supplemented with 1 mg/L of ceftriaxone incubated at 44°C, and a maximum of three colonies were subcultured and stored for further analysis (Agersø *et al.*, 2012).

The samples of five pooled cloacal swabs from broiler flocks were mixed in 3 mL of saline. Thereafter, 1 mL of suspension was transferred to 9 mL of MacConkey broth supplemented with 1 mg/L of ceftriaxone. The same procedure as described for sock samples was followed. *E. coli* was identified on CHROM Orientation agar (Becton Dickinson A/S, Brøndby, Denmark).

Detection of ESC genes and minimal inhibitory concentration (MIC) testing

The genetic background for ESC-producing *E. coli* was examined as previously described (Agersø *et al.*, 2012). In brief, all cephalosporinase-producing *E. coli* were initially tested by polymerase chain reaction (PCR) for $bla_{\text{CMY-2}}$. If negative, the isolates were subsequently tested for $bla_{\text{CTX-M}}$ genes, bla_{SHV} and bla_{TEM} genes by PCR and sequencing (Agersø *et al.*, 2012).

MIC were determined for $bla_{\text{CMY-2}}$-positive isolates for the following non-β-lactam antimicrobial agents: tetracycline (2–32 mg/L), chloramphenicol (2–64 mg/L), florfenicol (2–64 mg/L), sulfamethoxazole (64–1024 mg/L), trimethoprim (1–32 mg/L), apramycin (4–32 mg/L), gentamicin (0.5–16 mg/L), neomycin (2–32 mg/L), spectinomycin (16–256 mg/L), ciprofloxacin (0.015–4 mg/L), nalidixic acid (4–64 mg/L) by use of Sensititre (Trek Diagnostic Systems Ltd., West Sussex, UK), and following Clinical and Laboratory Standards Institute guidelines as previously described (CLSI, 2008). Resistance was determined by use of European Committee on Antimicrobial Susceptibility Testing epidemiological cutoff values (EUCAST, 2012). The *E. coli* strain ATCC 25922 was used for quality control.

Pulsed-field gel electrophoresis (PFGE), phylogrouping, and multilocus sequence typing (MLST) typing

All isolates positive for $bla_{\text{CMY-2}}$ (Table 1) were subtyped by use of PFGE with some modifications to the method described by Brolund *et al.* (Brolund *et al.,* 2010). In brief, the DNA was digested with *Xba*I at 37°C. The electrophoresis was performed with a CHEF DR III System (Bio-Rad Laboratories, Hercules, CA) using 1% SeaKem Gold (Lonza, Rockland, ME) agarose in 0.5 × Tris-borate-EDTA. Running conditions were 6 V/cm and included angle 120°C in 14°C Tris-borate-EDTA buffer, with pulse times linearly increased from 12 s initial switch time to 40 s final switch time for 20 h.

TABLE 1. DESCRIPTION OF THE CMY-2-PRODUCING *ESCHERICHIA COLI*

Origin	*PFGE type*	*No. of isolates*	*% Similarity within group*	*Subgroups with 100% similarity*	*Non-β-lactam resistance*	*Phylotype*
PF	1	1			None	A-1
PF	2	1			NAL	B2-2
BF	3	1			None	A1
Meat‘09	4	1			None	D1
PF, BF	5	2	80		SMX (1)	A-1
BF	6	1			None	A-1
PF, BF	7	2	97.1		None	A-2
PF	8	1			None	A-2
PF, BF	9	2	81.1		None	B1-1
Meat’09	10	1			TET	D2
PF	11	1			None	A-2
PF	12	1			TET	A-2
PF	13	5	91.7	1	TET (4)	A-1
Meat’10	14	1			None	NT
PF	15	1			None	A-2
BF	16	2	97.3		TET (1)	B1-1
BF	17	1			None	B1-1
Meat’10	18	2	98		None	D1
BF, meat’10	19	3	96		TET (3)	A-2
Meat’10	20	1			None	A-2
PF, BF, meat‘10	21	39	80.6	7	TET (1)	D2, NT(2)
PF	22	1			None	A1
PF	23	1			None	A2
BF	24	1			None	B1-1
BF	25	1			None	D2
BF	26	1			None	A-1
BF	27	3	97.6	1	None	B2-1
PF	28	1			None	A2
PF	29	1			None	A-2
BF	30	2	100		None	D1, D2
BF	31	1			TMP, NEO	A2
BF	32	1			None	A1
PF	33	1			None	A-2
PF	34	3	86.7	1	None	D2
PF	35	2	88.2		None	D2

PFGE type, pulsed-field gel electrophoresis patterns with less than 80% similarity; NAL, nalidixic acid; PF, parent farm; BF broiler flock; meat’09, broiler meat sampled in 2009; meat’10, broiler meat sampled in 2010; NEO, neomycin; SMX, sulfamethoxazole; TET, tetracycline; TMP, trimethoprim; NT, nontypeable.

*Xba*I digested DNA from *Salmonella* Braenderup H9812 was included as normalization standard on every gel.

PFGE analysis was performed using BioNumerics v. 4.61 (www.applied-maths.com) with the following settings: Dice band analysis, unweighted-pair group method with arithmetic mean dendrogram, optimization: 0.00%, position tolerance: 1.10%. The isolates were grouped into different groups if the compared PFGE patterns had less than 80% similarity.

Phylogrouping was performed on all bla_{CMY-2}-positive isolates as previously described (Clermont *et al.,* 2000). MLST was performed on selected isolates by use of whole genome sequencing and the web-server MLSTfinder (www.genomicepidemiology.org) as previously described (Larsen *et al.,* 2012).

Characterization of plasmid replicons and horizontal gene transfer

Selected isolates carrying bla_{CMY-2} were used as donors in filter-mating experiments to the recipient *E. coli* 1005RN, rif^R, nal^R (Table 2) as previously described (Agersø and Sandvang, 2005). Fifty microliters of the mating suspension was spread on one half of a Brain Heart Infusion agar supplemented with 1 mg/L ceftriaxone and 100 mg/mL of rifampicin, and on the other half the suspension was further spread with a loop to ensure single colonies of presumptive transconjugants. Suspected transconjugants were subcultured and checked for resistance to nalidixic acid. The presence of bla_{CMY-2} in the transconjugants was verified by PCR.

PCR-based replicon typing was used to characterize plasmids in the isolates chosen for mating experiments (Carattoli *et al.,* 2005). If no transconjugants were obtained, or if more than one replicon was found in a transconjugant, plasmids were purified and electroporated into *E. coli* 1005RN. Transformants were subjected to S1 nuclease PFGE to ensure the presence of a single plasmid as previously described (Bielak *et al*., 2011).

Consumption of aminopenicillin in the Danish broiler flocks

Data on consumption of aminopenicillins on the farm level was obtained from the VetStat database as previously described (Agersø *et al.*, 2012). Information on date of sale,

Table 2. Results of Mating Experiments, Multilocus Sequence Typing (MLST), and Replicon Typing of Selected *bla*$_{CMY-2}$ *Escherichia coli* Isolates

Donor ID	*Origin*	*MLST type*	*PFGE type*	*Replicon type*	*Non-β-lactam resistance*	*Phylotype*	*Transfer (yes/no) of* bla$_{CMY-2}$ *to* E. coli
2028-7	Parent flock	ST746 (single-locus variant)	1	IncI1		A1	Yes
2067-2	Parent flock	ST131	2	IncI1	NAL	B2-2	Yes
885-01	Meat	ST69	4	IncK		D1	Yes
5499-19	Broiler flock	ST746	5	IncI1	SMX	A1	Yes
7077-63	Broiler flock	ST115	6	IncI1		A1	Yes
2028-8	Parent flock	ST10	7	IncI1		A1	Yes
2115-5	Parent flock	ST616	9	IncI1		B1-1	Yes
1472-03	Meat	Nontypeable	10	IncK	TET	D2	No
2054-7	Parent flock	ST48	12	IncI1	TET	A2	Yes
2028-14	Parent flock	ST3272	13	IncI1	TET	A1	Yes
1061-1	Meat	ST10	14	IncI1		NT	Yes
5498-4	Broiler flock	ST212	16	IncI1		B1-1	Yes
7075-19	Meat	ST115	18	IncK		D1	Yes
7077-11	Broiler flock	ST23	19	IncK	TET	A2	Yes
7075-31	Meat	ST10	20	ND		A2	ND
2120-1	Parent flock	ST38	21	IncK		D2	Yes
7077-57	Broiler flock	ST38	21	IncK		D2	Yes
2028-10	Parent flock	ST1303	22	IncI1		A1	Yes
2054-3	Parent flock	ST1585	23	IncI1		A2	Yes
5499-28	Broiler flock	ST1056	24	IncI1		B1-1	Yes
5499-25	Broiler flock	ST1775	25	IncI1		D2	Yes
7077-7	Broiler flock	ST1594	26	IncK		A1	No
5498-25	Broiler flock	ST219	27	IncI1		B2-1	Yes
2054-5	Parent flock	ST1518	28	IncK		A2	Yes
2067-1	Parent flock	ST88	29	IncI1		A2	No
5499-9	Broiler flock	ST69	30	ND		D1	ND
7077-60	Broiler flock	ST410	31	IncI1		A1	Yes
2054-6	Parent flock	ST206	33	IncI1		A2	Yes
2123-1	Parent flock	ST350 (single-locus variant)	34	IncI1		D2	Yes
2028-4	Parent flock	ND	35	IncI1		D2	Yes

PFGE, pulsed-field gel electrophoresis; NT, nontypeable; ND, not determined; NAL; nalidixic acid; SMX, sulfamethoxazole; TET, tetracycline.

amount of drug prescribed, animal species, and code for farm identity (CHR number) was extracted from VetStat on November 7, 2012. The consumption of aminopenicillins in the broiler farms was defined as the consumption registered in the VetStat database for use in poultry on the given CHR number. The consumption was analyzed in two ways: (1) consumption of aminopenicillins at the farm at least once within the preceding 3 months prior to sampling, and (2) consumption of aminopenicillins at least once within the preceding 6 months prior to sampling.

Statistical analysis

Statistical significance tests of difference between proportions of samples positive for ESC-producing *E. coli* with or without use of aminopenicillins were calculated using chi-square, or Fisher exact test (two-tailed) when the number of positive samples was low (<5) (StatCalc in EpiInfo™ v. 6, Centers for Disease Control and Prevention, www.cdc.org). Estimation of exact 95% (two-sided) confidence intervals for proportions was based on binomial probability distributions as previously described (Armitage and Berry, 2001).

Results and Discussion

The Danish conventional broiler production is almost exclusively based on import of day-old parent animals from Sweden. In Sweden, day-old grandparent animals are imported from Scotland (P. Johanssen, DanHatch Ltd., personal communication). In Sweden, batches of imported day-old grandparent animals were found positive for ESC-producing *E. coli*, and the genotypes found were *bla*$_{CMY-2}$ and *bla*$_{CTX-M-1}$ (SVARM 2010, 2011).

In our study, cephalosporinase-producing *E. coli* were isolated from 93% (27/29) of the parent farms, from 27% (53/197) of the broiler flocks, and from 3.3% (4/121) and 8.6% (16/187) of Danish broiler meat sampled in 2009 and in 2010, respectively. The use of selective enrichment with ceftriaxone revealed ESC-producing *E. coli* in all the tested sample types. The method did not reveal the concentration of ESC-producing *E. coli* in the samples, and the infective concentration to humans is also unknown. The high prevalence of ESC-producing *E. coli* found in parent and broiler flocks was surprising, since cephalosporins have not been used in either the Danish or the Swedish broiler production, and in general the consumption of antimicrobials is low compared to other

production animals (DANMAP 2010, 2011; SVARM 2010, 2011). In January 2012, the British Poultry Association agreed to stop using cephalosporins in broiler production, but until then cephalosporins had been used in the United Kingdom (World Poultry, 2011; British Poultry Council, 2013). Therefore, the use of cephalosporins in the United Kingdom before 2012 may have selected for the ESC-producing *E. coli* occurring in the animals exported into Sweden and spread vertically from grandparents to parents imported into Denmark, but other (e.g., environmental) sources may also exist.

The most prevalent gene found to confer cephalosporinase-producing *E. coli* in our study was $bla_{\text{CMY-2}}$. This gene was found in all ESC-producing *E. coli* from parent flocks, in 89% (47/53) from broiler flocks, and in 50% (2/4) and 75% (12/16) from broiler meat in 2009 and 2010, respectively. Other genes were found in six isolates from broiler flocks ($bla_{\text{SHV-2}}$) and six isolates from broiler meat ($bla_{\text{CTX-M-1}}$), but not in parent flocks. Either they are present at a low level in the parent flocks or other routes of transmission such as cross-contamination or the outer environment exist. In other countries, large proportions of ESC-producing *E. coli* from broiler and broiler meat carry $bla_{\text{CMY-2}}$ are found, and due to the wide distribution to many countries of animals from the top of the breeding pyramid, it is suspected that the occurrence of cephalosporinase-producing *E. coli* may be due to the continuous introduction of imported animals carrying these bacteria (MARAN-2009, 2010; EFSA BIOHAZ, 2011; SVARM 2010, 2011). In Sweden, the same *E. coli* clones with $bla_{\text{CMY-2}}$ were found in imported grandparent animals and in all levels of the Swedish broiler production, indicating spread (Nilsson *et al*., 2014).

PFGE was performed on all $bla_{\text{CMY-2}}$-positive isolates in order to reveal whether the $bla_{\text{CMY-2}}$ isolates from parent flocks (29), broiler flocks (47), and broiler meat (14) were clonally related. Based on the PFGE patterns, the isolates grouped into 35 different PFGE groups with less than 80% similarity (Table 1, Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/fpd). One isolate from 29 of 35 groups was further MLST typed. Eleven groups consisted of 2 to 5 isolates with more than 80% similarity, and also subgroups with identical PFGE patterns were found within 4 of these groups. One group (PFGE type 21, Table 1, Supplementary Fig. S1) was interesting because this group contained 39 of 90 $bla_{\text{CMY-2}}$-positive isolates and these originated from parent flocks, broiler flocks, and broiler meat. Moreover, seven subgroups with 100% similar PFGE patterns between at least 2 isolates were found, and 1 of the subgroups contained isolates from parent flocks, broiler flocks, and meat (Table 1, Supplementary Fig. S1). This suggests that $bla_{\text{CMY-2}}$ spreads both horizontally and clonally in the production chain, and that some clones are more common than others. Therefore, ESC-producing *E. coli* originating from the animals may potentially reach the consumer as described for ESC-producing *Salmonella* Heidelberg in Canada (Dutil *et al.*, 2010). Similar clones and plasmids were also found in broilers, broiler meat, and patients in the Netherlands (Leverstein-van Hall *et al.*, 2011; Kluytmans *et al.*, 2013).

Phylogrouping of the $bla_{\text{CMY-2}}$-positive isolates showed phylotypes previously associated with disease in humans such as urinary tract infections (D and B2) and followed the PFGE type except for a few nontypeable isolates (Table 1) (Jakobsen *et al.,* 2010a, b; Johnson *et al.,* 2005). In the Netherlands, similar ESC-producing *E. coli* in poultry meat and from human infections has been described (Leverstein-van Hall, 2011; Kluytmans *et al*., 2013). So it is likely that some ESC-producing *E. coli* clones from the Danish broiler meat production cause infection in humans and should be further investigated. *E. coli* blood infections are mandatory for reporting in Denmark and may be MLST typed. *E. coli* MLST types formerly involved in human infection were found, including ST131, a global-spread human clone; ST48, recently found with $bla_{\text{CMY-2}}$ causing human clinical infection in Denmark; ST88, causing human infection; ST10, suspected to cause foodborne human infections; and the most dominant clone (43% of the isolates) ST38, also a type found in clinical *E. coli* (Table 2) (Guillouzouic *et al.,* 2009; Jørgensen *et al.,* 2010; Poirel *et al.*, 2011; Manges and Johnson, 2012). MLST types uncommon in human infections were also found, but these could be involved in horizontal spread of $bla_{\text{CMY-2}}$ to human pathogens.

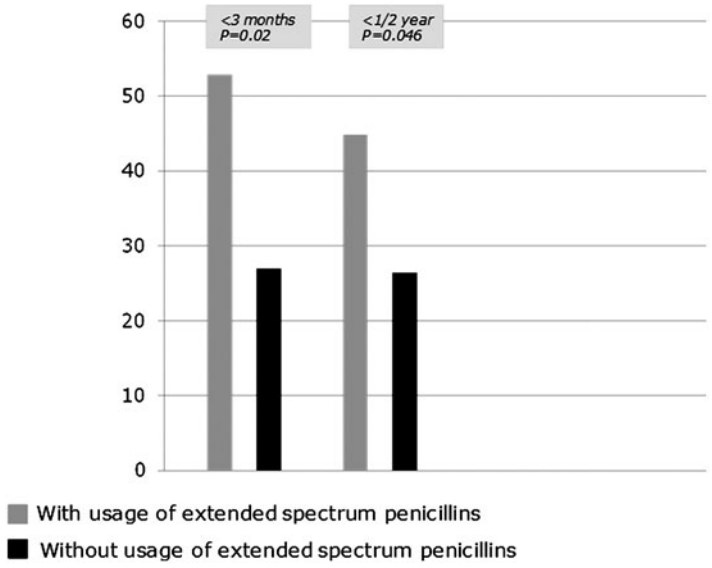

FIG. 1. Occurrence of extended-spectrum cephalosporinase-producing *Escherichia coli* in broiler flock with and without registered use of aminopenicillins on farm level up to 3 and 6 months prior to slaughter. The numbers on the *y*-axis represent the percentage of samples positive for ESC-producing *E. coli*.

Twenty-eight isolates representing 27 different PFGE types were used as donors in mating experiments, and 25 could transfer bla_{CMY-2} to an *E. coli* recipient (Table 2). Moreover, bla_{CMY-2} was found to be located on two types of plasmids: IncI1 (20) and IncK (8), so the presence of different clones can be explained by horizontal transfer of such plasmids, but may also be due to other introduction routes (e.g., from the outer environment, and contact to humans, insects, or animals). A study from Sweden found *E. coli* from broilers carrying bla_{CMY-2} on IncK plasmids, and *E. coli* isolates with bla_{CMY-2} carried by IncI1 or IncK plasmids have previously been found in humans, meat, and production animals (Börjesson *et al*., 2013). Moreover, bla_{CMY-2} has also been found to be associated with other plasmids such as IncI2 and IncA/C (Verdet *et al*., 2009; Antunes *et al.*, 2012; Börjesson *et al.,* 2013).

Susceptibility testing of the bla_{CMY-2} positive isolates showed most isolates being pansusceptible to all other non-β-lactam antimicrobials tested (Table 2). However, 11% (10/90) of the isolates were resistant to tetracyclines, the second most used antimicrobials in Danish broiler production. Single isolates were resistant to nalidixic acid, sulfamethoxazole, trimethoprim, and neomycin, respectively. As ESC-producing *E. coli* are resistant to aminopenicillins, we investigated whether ESC-producing *E. coli* more often originated from broiler farms that used aminopenicillins. One hundred eighty-eight of 197 flocks had information on farm origin. The flocks originated from 99 different farms. Seventeen and 29 flocks had registered use of aminopenicillins on farm level up to 3 and 6 months prior to slaughter, respectively. It was not possible to tell whether the aminopenicillins were used for the flock sampled, for a flock in another house, or for a flock previously raised in the same house, but a significantly higher occurrence of ESC-producing *E. coli* was observed in flocks from farms with use of aminopenicillin up to 3 months (52.9% [9/17], confidence interval [CI] [27.8–77.0%] vs. 26.9% [46/171] CI [20.4–34.2%], [$p = 0.02$]) and 6 months (44.8% [13/29], CI [26.4–64.3%] vs. 26.4% [42/159], CI [19.8–34.0%], [$p = 0.046$]) prior to slaughter (Fig. 1). Although the use of aminopenicillins is registered on farm level and not on flock level, the results indicate that aminopenicillins may have selected for ESC-producing *E. coli* and may thereby contribute to maintenance of ESC *E. coli* in the broiler flocks. Thus, the presence of ESC-producing *E. coli* on farms may be due to continuous introduction (e.g., with day-old parent chicks carrying ESC-producing *E. coli*), and/or due to carryover from one flock to the next and/or antimicrobial consumption. However, the relative importance of these factors is unclear, and carryover from litter or other environmental routes may also influence the presence of ESC-producing *E. coli*. Most of the broiler flocks (159) originated from farms with no use of aminopenicillins at least up to 6 months prior to slaughter, so the main reason for occurrence of ESC-producing *E. coli* is most likely the continuous introduction of animals, but a low fitness cost of ESC plasmids could also affect persistence.

In conclusion, ESC *E. coli* from imported parent animals spread clonally and horizontally even in a country with no use of cephalosporins in the poultry production. ESC-producing *E. coli* from imported animals is to some extent selected for by aminopenicillins. Therefore, the focus should be on reducing both use of cephalosporins and aminopenicillins. Even though *E. coli* carrying bla_{CMY-2} often is polyclonal, some clones, including MLST types involved in human infections, seem to establish better than others. Therefore, factors important for persistence and spread of ESC-producing *E. coli* should be investigated.

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Disclosure Statement

No competing financial interests exist.

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