Evaluación del uso de antimicrobianos como factor de riesgo relacionado con la aparición de resistencia a cefalosporinas en *Escherichia coli* y *Salmonella* en cerdos

Antimicrobial use as a risk factor associated with the emergence of cephalosporin resistance in *Escherichia coli* and *Salmonella* spp in pigs

Karla Cameron Veas

Doctorado en Medicina y Sanidad Animal

Departamento de Sanidad y Anatomía Animal

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Shedding of cephalosporin resistant *Escherichia coli* in pigs from conventional farms after early treatment with antimicrobials

Karla Cameron-Veas [a], Miguel A. Moreno [b,c], Lorenzo Fraile [d], Lourdes Migura-Garcia [a,*,1]

[a] Centre de Recerca en Sanitat Animal (CReSA) – Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Campus UAB, 08193 Barcelona, Spain
[b] Centro de Vigilancia Sanitaria Veterinaria, Universidad Complutense de Madrid, 28040 Madrid, Spain
[c] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, 28040 Madrid, Spain
[d] Departamento de Producción Animal, Universidad de Lleida, 25003 Lleida, Spain

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ABSTRACT

This study assessed the dynamics of cephalosporin resistant (CR) *E. coli* populations during the life cycle of pigs treated early in life with ceftiofur or tulathromycin. The study was conducted at eight conventional pig farms; four for each treatment with ceftiofur or tulathromycin. At each farm, 70 7-day-old piglets were divided into two groups: a control group ($n = 30$) and a treatment group ($n = 40$). Faecal samples were collected on day 0 and on days 2, 7 and 180 post-treatment. Sows were also sampled on day 0. CR *E. coli* were selected on MacConkey agar with ceftriaxone.

On five farms, 7-day-old piglets excreted CR *E. coli* before treatment associated with the presence of CR *E. coli* in sows. The occurrence of CR *E. coli* positive animals decreased with increasing piglet age. The remaining three farms tested negative for CR *E. coli* during the study period. Results demonstrated great variability in the frequency of CR *E. coli* positive animals between farms, independent of treatment. Treatment with ceftiofur resulted in a transitory increase in the counts of CR *E. coli* after 48 h. However, other risk factors including the presence of CR *E. coli* in sows and animal age were more important than antimicrobial treatment. Accordingly, intervention strategies targeting sows would likely have a beneficial effect in reducing the occurrence of antimicrobial resistance in primary pig production.

Introduction

One of the negative consequences of the extensive use of antimicrobials in veterinary medicine is the appearance of bacteria resistant to antimicrobials in food producing animals. In particular resistance to 3rd and 4th generation cephalosporins has increased over recent decades (Guardabassi, 2013). These drugs have been classified by the World Health Organization (WHO) and the World Organisation for Animal Health as critically important in both human and veterinary medicine (Collignon et al., 2009). Ceftiofur and cefquinome, 3rd and 4th generation cephalosporins, respectively, are licensed to treat food producing animals. Attempts have been made to quantify the contribution of resistant isolates causing human infections derived from food producing animals. Collignon et al. (2013) estimated that the use of antimicrobial drugs including cephalosporins used in poultry production has caused approximately 1518 human deaths in Europe over a 1-year period. However, this number is questionable since the authors did not consider other potential sources of spread of antimicrobial resistant bacteria into the community.

Although the use of antimicrobials in veterinary medicine is decreasing significantly across Europe due to the application of new programs (Schwarz et al., 2001; Garcia-Migura et al., 2014), there are many differences in antibiotic policy for treating animals in Europe. In the pig industry, antimicrobials are usually administered via feed or by water for metaphylaxis, which implies treatment of both sick and healthy animals (Burow et al., 2014). Additionally, other practices such as prophylaxis to prevent infection in specific risk situations (e.g., transportation in limited spaces) are ongoing (Laxminarayan et al., 2013). Although some countries such as Denmark have forbidden prophylactic use of antimicrobials, in other European countries it is common practice to administer prophylactic antimicrobials, such as ceftiofur, to piglets during the suckling period (L. Fraile, personal communication; Jorgensen et al., 2007; Callens et al., 2012). In Spain, beta-lactam antimicrobials (penicillins and cephalosporins) and macrolides (tulathromycin and tildipirosin) are the most commonly prescribed drugs during the suckling period (L. Fraile, personal communication).

The first step to control the emergence of antimicrobial resistance (AR) is to properly assess the selection pressure exerted by the use of these antimicrobials (Callens et al., 2012). In this context, the aim of this study was: (i) to evaluate if treatment with ceftiofur

* Corresponding author. Tel.: +34 93 581 32 84.
E-mail address: lourdes.migura@irta.cat (L. Migura-Garcia).
[1] Current address: Campus de la Universitat Autònoma de Barcelona, Edifici CReSA s/n, 08193 Cerdanyola del Vallès (Bellaterra), Spain.

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is a risk factor for the emergence of cephalosporin resistant *Escherichia coli* (CR *E. coli*) during the nursing period in conventional pig farms; and (ii) to assess if these farms are a reservoir of resistant bacteria that can persist and enter the food chain. Since tulathromycin is also administered for prophylaxis in some conventional farms, the third aim was to determine if treatment of suckling piglets with this macrolide is a risk factor for the emergence of CR *E. coli* due to the presence of co-resistance between different antimicrobial families. With the objective of analysing the dynamics of the CR *E. coli* population, faecal counts of CR *E. coli* in individual pigs receiving different treatments were monitored during medication and before slaughter.

Materials and methods

Study design

This study was performed in eight conventional farms located in the Northeast of Spain (Catalonia region). Inclusion criteria for the selection of these farms were the use of the same antimicrobials during the rearing cycle, no history of ceftiofur in the preceding 2 years and use of the same nutritional program, except for Farm 1, which used a different antimicrobial combination (Table 1). Seven of the eight farms belonged to a large farm integration system, with two different sources of gilts (designated as A and B) to maintain the breeding herd and piglet production (Table 1). The sampling period commenced in November 2012 and finished in May 2014. On each farm, seven sows in the last week of gestation were randomly selected and spatially separated in different farrowing rooms. After farrowing, litters were randomly allocated as "treated" or "untreated" and 70 7-day-old offspring (10 per mother) were randomly selected and ear tagged in both ears for identification. On each farm, piglets were divided into two groups: a control ($n = 30$) and a treated group ($n = 40$). The groups remained separated over the study period including during transportation of animals to the finishing farm, except for Farm 2 that presented a farrow to finish cycle. In four of the farms (Table 1), the treated group received 5 mg of ceftiofur/kg of body weight (bw) by single intramuscular (IM) injection (Naxcel, Zoetis Spain), whereas in the other four farms, the treated groups were administered 2.5 mg of tulathromycin/kg of bw by single IM injection (Draxxin, Zoetis Spain). Pigs were fed using a standard nutritional program set by the companies, which included use of different prophylactic antimicrobials during the nursery period (Table 1). This treatment commenced after the administration of ceftiofur or tulathromycin (from 21 to 70 days of age). In five farms, sows received a treatment of 20 mg of oxytetracycline/kg of bw/day in feed during the last 2 weeks of the gestation period (Table 1).

The 7-day-old piglets were individually swabbed and faecal content was collected into a sterile tube before treatment with ceftiofur or tulathromycin on day 0. Further samples were collected on days 2 and 7 post-treatment. On day 0, faecal samples were also collected from the mothers. A final sampling was performed before the animals departed to the slaughterhouse to determine the presence of resistant bacteria (at approximately 180 days of life). Sampling points were selected based on a previous longitudinal study performed by our research group (Cameron-Veas et al., 2015) that demonstrated a significant increase in the selection of CR *E. coli* 48 h after ceftiofur treatment. During the course of the study, a total of 164 animals were not sampled at some point due to either death or loss of ear tags. Of those, 23 belonged to Farm 8 and were sent to the abattoir before they could be sampled.

Isolation, identification and quantification of E. coli

Faecal samples were transported at 4 °C on the day of sampling to the laboratory after which a primary culture was performed by plating a loopful of homogenized faeces on MacConkey agar supplemented with ceftriaxone (1 mg/L), followed by overnight incubation at 45 °C. With the aim of measuring changes in the resistant population before, during treatment and at the end of the rearing period, enumeration of CR *E. coli* was performed in all samples positive for CR *E. coli* in the primary culture. For quantification of CR coliforms, 1 g of homogenized faeces was suspended in 9 mL of Phosphate Buffer Saline (PBS), followed by serial 10-fold dilutions (from 10^{-1} to 10^{-6}). Dilutions of 10^{-1}–10^{-3} were plated on MacConkey agar supplemented with ceftriaxone (1 mg/L). Dilutions of 10^{-4}–10^{-6} were plated on MacConkey agar without antibiotics to account for the total *E. coli* population. Only lactose positive colonies were counted. *E. coli* isolates were selected based on colony morphology. Three isolates were frozen per positive sample and one was confirmed to be *E. coli* by PCR methods (Heininger et al., 1999).

Statistical analyses

All statistical analyses were carried out using the SAS System V.9.1.3 (SAS Institute). Individual pigs were used as the experimental unit unless the farm was the experimental unit as detailed below. Analyses took into account that a pig was sampled several times (repeated measures) as well as the cluster effect due to the sow. The significance level (P) was set at 0.05 with statistical tendency reported when $P \leq 0.10$. Pigs were classified as CR *E. coli* positive if they had at least one isolate phenotypically confirmed. Farms were classified as CR *E. coli* positive if they had one positive animal during the study period. The total *E. coli* and CR *E. coli* counts were expressed as colony formation units/g of faeces (CFU/g) and analysed as decimal logarithms.

Different statistical analyses were performed with the data obtained from this study. The first one comprised descriptive statistics based on contingency tables (χ^2) to evaluate at farm level the relationship between a piglet being positive for CR *E. coli* before antimicrobial treatment and four of the following nominal variables: farm, origin of the sows (A or B), sow facilities (open pen or boxes) and antimicrobial treatment given to the sows (use or no use of oxytetracycline). Proportions of pigs shedding CR *E. coli* were estimated for each sampling point on each farm, and the proportion of pigs changing carriage status between different sampling times was calculated and compared using a χ^2 test.

An exact logistic regression was used to calculate the probability of a pig to be colonized with CR *E. coli* at the time of slaughter. A variable was defined to indicate the presence or absence (1 and 0, respectively) of CR *E. coli* in each animal at slaughter time. This variable was used as a response variable to establish the effect of the different covariates defined as: positivity of the sow at the first sampling time and the antibiotic treatment applied to the piglets. For this analysis, the litter effect (hierarchical structure) was accounted for as a random effect.

Mean logs of positive CR *E. coli* counts per farm (1–8), antibiotic treatment (control, ceftiofur and tulathromycin) and sampling times (0, 2, 7, and 180 days post-treatment) were calculated and compared using a parametric analysis (ANOVA test). Furthermore, a final statistical model analysed the dynamics of the mean counts of CR *E. coli* in piglets depending on the farm, piglets' age and antimicrobial treatment using a zero-inflated negative binomial regression model. An interaction term between piglets-age group and farm was included to assess if counts differed between age groups in different farms. This model took into account the hierarchical structure and the repeated measure effect of sampling the same animals at different points in time.

Table 1
Farm characteristics including production type, origin of sows, type of pen, usage of antimicrobials and treatment administered during the study of conventional pig farms in Catalonia, Spain from November 2012 to May 2014.

Farm	Origin of sows	Production	Treatment of sows (oxytetracycline)[†]	Type of pen	Antimicrobials orally administered in-feed to piglets during the rearing period (mg of antibiotic/kg of body weight/day)			Piglet experimental treatment
					Pre-starter (21–35 days postpartum)	Starter 1 (35–49 days postpartum)	Starter 2 (49–70 days postpartum)	
1[*]	Unknown	Phase 1	No	Open	15 mg of amoxicillin (Zoobiotic Globulit®, Laboratorios Calier SA), 5 mg of colistin sulphate (Laboratorios Andersen SA)			Tulathromycin
2	A	Farrow to finish	Yes	Open	15 mg of amoxicillin (Zoobiotic Globulit®, Laboratorios Calier SA), 10 mg of apramycin (Apralan®, Laboratorios Elanco SA)	10 mg of apramycin (Apralan® Laboratorios Elanco SA), 4 mg of tiamulin (Nemutin Premix, SP Veterinaria SA), 20 mg of oxytetracycline (Oxitetraciclina Maymó, Laboratorios Maymó SA)	4 mg of tiamulin (Nemutin Premix, SP Veterinaria SA), 20 mg of oxytetracycline (Oxitetraciclina Maymó, Laboratorios Maymó SA)	Ceftiofur
3	B	Phase 1/2	Yes	Boxes				Tulathromycin
4	B	Phase 1/2	No	Open				Ceftiofur
5	B	Phase 1/2	Yes	Open				Ceftiofur
6	A	Phase 1	No	Open				Tulathromycin
7	A	Phase 1	Yes	Open				Tulathromycin
8	B	Phase 1	Yes	Boxes				Ceftiofur

Phase 1/2, phases 1 and 2; Phase 1, up to 6 kg piglet; Phase 2, up to 20 kg of body weight.
[*] Farm 1 belonged to a different integration company.
[†] 20 mg of oxytetracycline/kg of bw/day in feed during the last 2 weeks of the gestation period.

Table 2
Percentage of animals (sows, piglets and finishers) per farm from the treated and control groups shedding CR *E. coli* during each of the sampling times.

Farm	Treatment	Positive sows	Day 0 prior treatment		2 days post-treatment		7 days post-treatment		Departure to abattoir	
			Control	Treated	Control	Treated	Control	Treated	Control	Treated
1	Tulathromycin	5/7	5/30 (17%)	30/40 (75%)	9/30 (30%)	26/40 (65%)	2/28 (7%)	32/39 (82%)	0/23	0/35
2	Ceftiofur	7/7	28/30 (93%)	37/40 (93%)	27/30 (90%)	36/40 (90%)	23/30 (77%)	34/38 (89%)	1/21 (5%)	18/30 (60%)
3	Tulathromycin	0/7	0/30	0/40	0/30	0/40	0/30	0/40	0/24	0/36
4	Ceftiofur	6/7	22/29 (76%)	27/39 (69%)	22/29 (76%)	31/39 (79%)	25/29 (86%)	32/39 (82%)	0/17	0/35
5	Ceftiofur	0/7	0/30	0/40	0/30	0/40	0/26	0/37	0/17	0/33
6	Tulathromycin	5/7	21/30 (70%)	32/40 (80%)	27/30 (90%)	32/40 (80%)	23/30 (77%)	30/40 (75%)	5/24 (21%)	8/34 (24%)
7	Tulathromycin	6/7	26/30 (87%)	21/40 (53%)	24/30 (80%)	31/40 (78%)	23/30 (77%)	28/40 (70%)	8/16 (50%)	14/30 (47%)
8	Ceftiofur	0/7	0/29	0/37	0/29	0/36	0/23	0/33	0/0*	0/21

* Animals from the control group were not present on the farm at this sampling time.

Results

Presence of CR E. coli

The occurrence of CR *E. coli* was extremely variable among farms, ranging from 0% to 93% of animals positive for CR *E. coli* (Table 2). Detection of CR *E. coli* showed a statistical tendency to be positive in sows descending from gilts of A origin compared to B origin sows ($P = 0.10$), and in sows allocated in pens rather than in boxes ($P = 0.10$). In all farms where sows were shedding CR *E. coli* offspring were also excreting CR *E. coli* before any treatment was administered. In three of the farms where CR *E. coli* were not detected in any of the sows, they were also not detected in their progeny. The detection of positive CR *E. coli* piglets before treatment was significantly associated with the presence of positive CR *E. coli* sows at a farm level ($P = 0.02$). The detection of positive CR *E. coli* in sows was not associated with the administration of oxytetracycline ($P > 0.05$) during the gestation period (Table 4).

Overall, there were no significant differences in the proportions of animals shedding CR *E. coli* between control and treated groups (ceftiofur or tulathromycin) throughout the trial ($P > 0.05$), except on two occasions. In Farm 2 (Table 2), the proportion of animals shedding CR *E. coli* in the group treated with ceftiofur before slaughter was significantly higher than in the control group ($P < 0.05$). For Farm 1, the proportion of animals shedding CR *E. coli* in the group treated with tulathromycin was significantly higher ($P < 0.05$) than in the control group at 0, 2 and 7 days post-treatment. Interestingly, CR *E. coli* could not be isolated from Farm 1 pigs at the end of the study period.

The isolation of CR *E. coli* decreased significantly ($P < 0.05$) with the age of the animals in all of the studied farms positive for CR *E. coli* from the first week of life (sampling 0, 2 and 7 days post-antibiotic treatment) to the last sampling point (previous to slaughter). No significant increase was observed in the proportion of piglets positive for CR *E. coli* during the first week after use of ceftiofur or tulathromycin. The presence of CR *E. coli* in pigs before slaughter was observed only in three of the eight farms, with 48%, 37% and 22% of animals positive for CR *E. coli*. In these three farms, the proportion of piglets with CR *E. coli* before applying any antimicrobial treatment was higher than 67%.

The logistic regression analysis demonstrated that pigs were more likely to be positive for CR *E. coli* at slaughter if they had been positive as piglets during the first week of life (odds ratio [OR] = 3.5, 95% confidence interval [CI] = 1.6–8.4; $P = 0.001$) and if their mothers were positive for CR *E. coli* (OR = 4.9, 95% CI = 2.02–13.9; $P = 0.0002$). Antibiotic treatment administered to piglets did not increase the odds ($P > 0.05$) of being CR *E. coli* positive at slaughter (Table 4).

Faecal counts of CR E. coli

The counts of CR *E. coli* (Table 3) were significantly different among farms ($P < 0.05$) at days 2 and 7 post-treatment, and showed a statistical tendency ($P = 0.09$) at the slaughterhouse. Similar results were observed for total *E. coli* population counts. Counts of CR *E. coli* at days 2 and 7 post-treatment were significantly higher ($P < 0.05$) in animals treated with ceftiofur than in animals treated with tulathromycin or in animals from the control group. For the total *E. coli* population, a significant increase in counts was observed in CR *E. coli* positive piglets only after 2 days post-treatment ($P < 0.05$). Finally, CR *E. coli* counts obtained from animals prior to departure to the slaughterhouse were significantly lower ($P < 0.05$) than those obtained during the first week of life.

When the dynamics of the mean counts of CR *E. coli* in piglets were analysed by a zero-inflated negative binomial regression model, taking into account all variables, the counts of CR *E. coli* were significantly associated ($P < 0.05$) with farm (very variable among farms) and the age of the animals; how older the animal, how lower the counts of CR *E. coli*. There was a significant interaction ($P < 0.05$) between the piglets' age and the antibiotic treatment received during the first week of life; thus, the treatment with ceftiofur increased the counts of CR *E. coli* in positive animals during this short period of time after treatment. Finally, there was also a significant interaction ($P < 0.05$) between the farm and the age of the piglet, meaning that the variation in counts at each sampling time differed depending on the farm (Table 4).

Table 3
Mean of log counts (natural logarithm) of faecal CR *E. coli* per farm, groups (treated and control) and sampling time.

Farm*	Treatment	Positive sows	Day 0 prior treatment		48 h post-treatment		7 days post-treatment		Departure to abattoir	
			Control	Treated	Control	Treated	Control	Treated	Control	Treated
1	Tulathromycin	5/7	4.77 (5)	4.52 (30)	4.17 (9)	3.69 (26)	4.42 (2)	4.43 (32)	–	–
2	Ceftiofur	7/7	5.50 (28)	4.87 (37)	5.51 (27)	6.28 (36)	5.85 (23)	6.22 (34)	3.35 (1)	3.16 (18)
4	Ceftiofur	6/7	4.28 (22)	4.43 (27)	4.49 (22)	4.65 (31)	4.90 (25)	5.51 (32)	–	–
6	Tulathromycin	5/7	5.50 (21)	5.18 (32)	5.50 (27)	5.12 (32)	4.74 (23)	4.28 (30)	3.34 (5)	3.13 (8)
7	Tulathromycin	6/7	5.44 (26)	4.81 (21)	4.94 (24)	4.89 (31)	4.68 (23)	4.76 (28)	3.80 (8)	3.97 (14)

In brackets, number of CR *E. coli* positive samples with valid counts.
* Farms 3, 5 and 8 were negative for CR *E. coli*.

Table 4
Summary of the main statistical analyses carried out in this research work.

Variables	The relationship between a piglet being positive for CR *E. coli* before antimicrobial treatment		
	Significant	*P*	Observation
Farm	Yes	$P = 0.02$	–
Origin of sows (A or B)	Statistical tendency	$P = 0.10$	–
Sow facilities (open pen or boxes)	Statistical tendency	$P = 0.10$	–
Treatment with oxytetracycline during the gestation period	No	$P > 0.05$	–
Variables	**The probability of a pig to be colonized with CR *E. coli* at the time of slaughter**		
	Significant	*P*	Odds ratio and 95% confidence interval
Positivity of the sow at first sampling time	Yes	$P = 0.0002$	4.9 (2.02–13.9)
Positivity of the piglet during the first week of life	Yes	$P = 0.001$	3.5 (1.6–8.4)
Antimicrobial treatment to piglets	No	$P > 0.05$	NA
Variables	**The dynamics of counts of CR *E. coli* in piglets during the whole rearing period**		
	Significant	*P*	Observation
Farm	Yes	$P = 0.01$	–
Age of the piglets	Yes	$P = 0.02$	Significant interaction between the age of the piglet and the farm
Antimicrobial treatment to piglets	No	$P > 0.05$	Significant interaction between the age of the piglet and the antibiotic treatment during the first week of life

NA, not applicable.

Discussion

Antimicrobial resistance is a complex subject and there are limited studies describing the relationship between antibiotic treatment and resistance over time (Mathew et al., 2005). Most AR studies are designed to associate the effect of antimicrobial treatments in a cross-sectional manner (Jorgensen et al., 2007; Cavaco et al., 2008; Agerso et al., 2012). These studies measured the presence of AR and tried to associate it with use of a particular antimicrobial without considering the age of the animals sampled, or the different management practices of each farm (e.g., nutritional programs, breeding sources) that may influence the final outcome. Our study has demonstrated that in farms where sows were free of CR *E. coli*, the administration of ceftiofur to 7-day-old piglets did not seem to pose sufficient selection pressure to select for ceftiofur-resistant organisms. However, those farms with piglets initially shedding CR *E. coli* at day 0 did experience an increase in the percentage of animals excreting CR *E. coli* during pre-weaning, independent of the treatment. By the end of the life cycle the proportion shedding CR *E. coli* had decreased. It appears that the initial load of resistant bacteria depends mainly on the farm and that there is a time effect that reduces the proportion of animals positive for CR *E. coli* as well as the counts of CR *E. coli* in individual animals. Similar dynamics have been described for CR *E. coli* in other studies (Jorgensen et al., 2007; Singer et al., 2008; Cameron-Veas et al., 2015), and may be explained by changes in the composition of the intestinal microbiota of pigs over time (Katouli et al., 1995; Hansen et al., 2014).

It remains unclear why Farm 1 had such a distribution of CR *E. coli* positive animals with the highest proportions of CR *E. coli* in the tulathromycin treated group from day 0 and increasing proportions up to day 7. We could not find a plausible explanation, since the sows were selected randomly. A recent study suggested that there is a reduction in prevalence and diversity of CR *E. coli* strains after weaning (Hansen et al., 2014), and results from our study may reflect similar dynamics. Additionally, differences in sow origin or management such as the use of colistin on this farm may account for the difference observed in comparison with the other farms. However, counts of CR *E. coli* did not increase during treatment with tulathromycin as observed in those farms treated with ceftiofur, where a significant increase was observed. It is noteworthy that by the time of slaughter, we could not detect CR *E. coli* in any of the animals, nor from control either from the treated group.

No antibiotic selection pressure was needed to detect CR *E. coli* in 5/8 farms, given that 7-day-old piglets were excreting resistant bacteria before any treatment was administered. Based on these results, it appears that these bacteria were circulating in the farms prior to farrowing. The fact that most of the sows were positive for CR *E. coli* might explain the presence in their offspring due to vertical transfer during weaning (Hansen et al., 2013; Liebana et al., 2013; Callens et al., 2015). Additionally, sows originating from A gilts had a tendency to harbor CR *E. coli*, suggesting circulation of the same resistant organisms within these integrated production systems.

At the abattoir pigs carrying CR *E. coli* represent a public health concern (Ramos et al., 2013). Faecal counts up to 10^4 cfu/mg of faeces were observed in some of the pigs in this study. Although a reduction in counts is expected during meat processing, some processes at the slaughterhouse may contribute to contamination of meat with resistant bacteria or to the cross-contamination of clean animals (Gomes-Neves et al., 2014). As a consequence, CR *E. coli* contaminated meat may reach the consumer. Infective doses for humans with *E. coli* may vary between 10^3 and 10^8 cfu. It is not clear what dosage of CR *E. coli* is required to transfer resistance genes to the human microbiota and/or to colonize the human gut (Smet et al., 2011). Any effort contributing to reduction of the load of antimicrobial resistant bacteria in primary production will have an impact in the final product, and therefore in protecting human health.

In this study pig production companies applied several families of prophylactic antimicrobials during the nursery period. Although such usage was not recommended by the authors of this research work, it allowed for the investigation of the emergence of CR *E. coli* in animals receiving registered doses of a combination of different antimicrobials (beta-lactams, aminoglycosides, tetracycline and pleuromutilins). Thus, it could be considered a worst-case scenario for the appearance of any AR that cannot be ignored when interpreting the results. Both groups in our study (control or treated) received the same antimicrobial selective pressure for at least 5 weeks. In spite of this long period of treatment, the proportion and counts of CR *E. coli* decreased from young piglets until the age of slaughter in all the farms. This result suggests that this treatment did not pose sufficient selection pressure to increase CR *E. coli* populations. In any case, it must be highlighted that we were unable to detect any CR *E. coli* throughout the trial in pigs coming from three out of eight farms that were negative at the first sampling time. However, we only focused on measuring CR *E. coli* and

other resistance phenotypes that this prophylactic treatment might have selected for were outside the scope of this study.

There are several limitations in this study. Due to the load of work, the number of farms included in the study was relatively low; however, there is sufficient power in the sample size to detect meaningful differences between the proportion of animals positive for CR *E. coli* and the counts of CR *E. coli* in animals treated with ceftiofur/tulathromycin and animals from the control group. Additionally, when performing experiments in real conventional farms, there are many factors that can also influence the results (e.g. sow origin, management, pig flow). Our experimental design does not have enough statistical power to detect significant differences for the effect of all the potential factors on the emergence of CR *E. coli*. In summary, we have described factors influencing the appearance of CR *E. coli* in pigs from conventional farms after early treatment with antimicrobials, but the influence of other factors, not described in this study, cannot be excluded.

Conclusions

This study has demonstrated a great variability in the frequency of CR *E. coli* between farms independent of the treatment. Results revealed a short time period when the treatment with ceftiofur resulted in a transitory increase in the shedding of CR *E. coli*. More importantly, other risk factors such as the presence of CR *E. coli* in the sows and the age of the animals at study have an important role in the persistence of CR *E. coli*. CR *E. coli* positive sows were more likely to have positive offspring, and CR *E. coli* positive piglets were likely to remain CR positive until slaughter. Strategies to control CR *E. coli* in the sows may prevent colonization of piglets by resistant bacteria at the beginning of the life cycle, which appears to be the critical point in time to reduce occurrence at slaughter.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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References

Agerso, Y., Aarestrup, F.M., Pedersen, K., Seyfarth, A.M., Struve, T., Hasman, H., 2012. 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. Journal Antimicrobial Chemotherapy 67, 582–588.

Burow, E., Simoneit, C., Tenhagen, B.A., Kasbohrer, A., 2014. Oral antimicrobials increase antimicrobial resistance in porcine *E. coli* – A systematic review. Preventive Veterinary Medicine 113, 364–375.

Callens, B., Persoons, D., Maes, D., Laanen, M., Postma, M., Boyen, F., Haesebrouck, F., Butaye, P., Catry, B., Dewulf, J., 2012. Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig herds. Preventive Veterinary Medicine 106, 53–62.

Callens, B., Faes, C., Maes, D., Catry, B., Boyen, F., Francoys, D., de Jong, E., Haesebrouck, F., Dewulf, J., 2015. Presence of antimicrobial resistance and antimicrobial use in sows are risk factors for antimicrobial resistance in their offspring. Microbial Drug Resistance 21, 50–58.

Cameron-Veas, K., Sola-Gines, M., Moreno, M.A., Fraile, L., Migura-Garcia, L., 2015. Impact of the use of beta-lactam antimicrobials on the emergence of *Escherichia coli* isolates resistant to cephalosporins under standard pig-rearing conditions. Applied Environmental Microbiology 81, 1782–1787.

Cavaco, L.M., Abatih, E., Aarestrup, F.M., Guardabassi, L., 2008. Selection and persistence of CTX-M-producing *Escherichia coli* in the intestinal flora of pigs treated with amoxicillin, ceftiofur, or cefquinome. Antimicrobial Agents Chemotherapy 52, 3612–3616.

Collignon, P., Powers, J.H., Chiller, T.M., Aidara-Kane, A., Aarestrup, F.M., 2009. World Health Organization ranking of antimicrobials according to their importance in human medicine: A critical step for developing risk management strategies for the use of antimicrobials in food production animals. Clinical Infection Diseases 49, 132–141.

Collignon, P., Aarestrup, F.M., Irwin, R., McEwen, S., 2013. Human deaths and third-generation cephalosporin use in poultry, Europe. Emerging Infectious Diseases 19, 1339–1340.

Garcia-Migura, L., Hendriksen, R.S., Fraile, L., Aarestrup, F.M., 2014. Antimicrobial resistance of zoonotic and commensal bacteria in Europe: The missing link between consumption and resistance in veterinary medicine. Veterinary Microbiology 170, 1–9.

Gomes-Neves, E., Antunes, P., Manageiro, V., Gartner, F., Canica, M., da Costa, J.M., Peixe, L., 2014. Clinically relevant multidrug resistant *Salmonella enterica* in swine and meat handlers at the abattoir. Veterinary Microbiology 168, 229–233.

Guardabassi, L., 2013. Sixty years of antimicrobial use in animals: What is next? Veterinary Records 173, 599–603.

Hansen, K.H., Damborg, P., Andreasen, M., Nielsen, S.S., Guardabassi, L., 2013. Carriage and fecal counts of cefotaxime M-producing *Escherichia coli* in pigs: A longitudinal study. Applied Environmental Microbiology 79, 794–798.

Hansen, K.H., Bortolaia, V., Damborg, P., Guardabassi, L., 2014. Strain diversity of CTX-M-producing *Enterobacteriaceae* in individual pigs: Insights into the dynamics of shedding during the production cycle. Applied Environmental Microbiology 80, 6620–6626.

Heininger, A., Binder, M., Schmidt, S., Unertl, K., Botzenhart, K., Doring, G., 1999. PCR and blood culture for detection of *Escherichia coli* bacteremia in rats. Journal Clinical Microbiology 37, 2479–2482.

Jorgensen, C.J., Cavaco, L.M., Hasman, H., Emborg, H.D., Guardabassi, L., 2007. Occurrence of CTX-M-1-producing *Escherichia coli* in pigs treated with ceftiofur. Journal Antimicrobial Chemotherapy 59, 1040–1042.

Katouli, M., Lund, A., Wallgren, P., Kuhn, I., Soderlind, O., Mollby, R., 1995. Phenotypic characterization of intestinal *Escherichia coli* of pigs during suckling, postweaning, and fattening periods. Applied Environmental Microbiology 61, 778–783.

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A.K., Wertheim, H.F., Sumpradit, N., Vlieghe, E., Hara, G.L., Gould, I.M., Goossens, H., et al., 2013. Antibiotic resistance-the need for global solutions. Lancet Infectious Disease 13, 1057–1098.

Liebana, E., Carattoli, A., Coque, T.M., Hasman, H., Magiorakos, A.P., Mevius, D., Peixe, L., Poirel, L., Schuepbach-Regula, G., Torneke, K., et al., 2013. Public health risks of enterobacterial isolates producing extended-spectrum beta-lactamases or AmpC beta-lactamases in food and food-producing animals: An EU perspective of epidemiology, analytical methods, risk factors, and control options. Clinical Infectious Diseases 56, 1030–1037.

Mathew, A.G., Garner, K.N., Ebner, P.D., Saxton, A.M., Clift, R.E., Liamthong, S., 2005. Effects of antibiotic use in sows on resistance of *E. coli* and *Salmonella enterica* Typhimurium in their offspring. Foodborne Pathogens Disease 2, 212–220.

Ramos, S., Silva, N., Canica, M., Capelo-Martinez, J.L., Brito, F., Igrejas, G., Poeta, P., 2013. High prevalence of antimicrobial-resistant *Escherichia coli* from animals at slaughter: A food safety risk. Journal Science Food Agriculture 93, 517–526.

Schwarz, S., Kehrenberg, C., Walsh, T.R., 2001. Use of antimicrobial agents in veterinary medicine and food animal production. International Journal Antimicrobial Agents 17, 431–437.

Singer, R.S., Patterson, S.K., Wallace, R.L., 2008. Effects of therapeutic ceftiofur administration to dairy cattle on *Escherichia coli* dynamics in the intestinal tract. Applied Environmental Microbiology 74, 6956–6962.

Smet, A., Rasschaert, G., Martel, A., Persoons, D., Dewulf, J., Butaye, P., Catry, B., Haesebrouck, F., Herman, L., Heyndrickx, M., 2011. In situ ESBL conjugation from avian to human *Escherichia coli* during cefotaxime administration. Journal Applied Microbiology 110, 541–549.