The Veterinary Journal 211 (2016) 21–25

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

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

ARTICLE INFO

Article history:
Accepted 27 February 2016

Keywords:
Ceftiofur
Tulathromycin
Cephalosporins
E. coli
Pigs

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.

http://dx.doi.org/10.1016/j.tvjl.2016.02.017
1090-0233/

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.