Voluntary ban on cephalosporin use in Danish pig production has effectively reduced extended-spectrum cephalosporinase-producing Escherichia coli in slaughter pigs

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Objectives: To measure the effect of a voluntary ban on cephalosporin usage in the Danish pig production on the prevalence of extended-spectrum cephalosporinase (ESC)-producing Escherichia coli in pigs and pork.

Methods: Data on cephalosporin consumption were obtained from the VetStat database. For detection of ESC-producing E. coli, three sampling types were included: at slaughter, caecal samples were collected from pigs in 2009 and 2010 (June) before and in two periods (2010 and 2011) after a voluntary ban on cephalosporins was effected (July 2010); at farm level, pools of five stool samples from different pigsties were collected in 2010 and in 2011; and samples from pork were collected randomly at retail stores and outlets from 2009 to 2011. ESC-producing E. coli was isolated after selective enrichment in MacConkey broth with 1 mg/L ceftriaxone. ESC genes were detected using PCR, microtube array and sequencing.

Results: From July 2010 the consumption of cephalosporins approximated zero. The occurrence of ESC-producing E. coli in pigs at slaughter was not significantly different (\( P = 0.7 \)) between 2009 [10.8% (85/786)] and 2010 [11.8% (48/407)], but in 2011 the occurrence \( [3.6\% (28/777)] \) decreased significantly \( (P < 0.001) \). A significant decrease \( (P = 0.002) \) in occurrence of ESC-producing E. coli at pig farm level from 2010 \( [11\% (11/99)] \) to 2011 \( (0/78) \) was also observed. The \( \text{bla}_{\text{CTX-M-1}} \) gene was most often detected \( (63\%) \), but \( \text{bla}_{\text{CTX-M-14}} \) and \( \text{bla}_{\text{CTX-M-15}} \) were also found. Occurrence in pork was between 1.3% and 0.9%.

Conclusions: The discontinuation of an already low use of cephalosporins in pig production has significantly reduced the occurrence of ESC-producing E. coli.

Keywords: swine, ESBL, ESC, CTX-M, antibiotic, consumption

Introduction

While several studies have shown that the use of antimicrobial agents will lead to increased occurrence of antimicrobial resistance, there is limited scientific evidence on the effects of reducing their usage. Discontinuation of the consumption of antimicrobial agents for growth promotion in production animals in Denmark has shown a large effect on the reduction of resistance to the antimicrobial agent,\(^1\) and changing levels of cefotiofur use in hatcheries in Quebec, Canada, resulted in changes in cefotiofur resistance in chicken Salmonella Heidelberg and Escherichia coli isolates, as well as human Salmonella Heidelberg isolates during the study period.\(^2\)

Currently, resistance to third- and fourth-generation cephalosporins in particular is considered a major public health threat.\(^3\) In Denmark, the consumption of third- and fourth-generation cephalosporins (mainly cefotiofur and ceftuquione) in pig production increased rapidly from 2001, reaching \( \sim 1\% \) of the total consumption in 2007–08 (129–128 kg of active compound).\(^4,5\) Despite this relatively low consumption, the use has been widespread in breeding herds, accounting for 83% and 87% of cephalosporin usage in pigs in 2007 and 2008, respectively.\(^4\) In breeding herds, third- and fourth-generation cephalosporins have been used commonly for prophylactic treatment of umbilical infection in piglets as one injection on the first or second day after birth.\(^6\) In 2007, the breeding farms that used cephalosporins produced 14% of the piglets produced in Denmark and in these farms the consumption corresponded to 3.5 defined animal daily doses (ADDs) (based on the dose for a 2 kg pig) per piglet produced.\(^6\) The amount used in breeding herds in 2008 corresponds to treatment of 6 186 667 piglets for 3 days (standard treatment 3 mg/kg for 3 days).\(^6\) Thus, a
large proportion of Danish pig production has been exposed to cephalosporins.

In Danish livestock, the first extended-spectrum cephalosporinase (ESC) producing isolates were detected among isolates from diagnostic submissions in 2005 and 2006. The first screening of ESC-producing E. coli was performed in 2009 and showed an increased level of ESC-producing E. coli among slaughter pigs treated with third- or fourth-generation cephalosporins at least once up to 1 year before slaughter.

From 2008 to 2009 the consumption of third- or fourth-generation cephalosporins decreased by 25%, and in July 2010 the Danish Agriculture and Food Council enforced a voluntary ban on cephalosporins for use in the pig production; since then there has been almost no usage of cephalosporins.

The aim of this study was to measure the effect of the voluntary ban in 2010 on the prevalence of ESC-producing E. coli in pigs and pork.

Materials and methods

Data on consumption

Data on consumption were obtained from the VetStat database. In Denmark, all therapeutic drugs are prescription only and reporting to the VetStat database of medicines prescribed for animals has been mandatory since 2001. Data on consumption in pigs are available from the Danish Veterinary and Food Administration (DVFA) homepage on a monthly basis.

Collection of samples

For detection of ESC-producing E. coli, samples were collected from pigs at slaughter, pigs at farm level and Danish retail pork.

Sampling of pigs at slaughter

Cæcal samples were collected from pigs from February to November 2009 and in June 2010 before a voluntary ban on cephalosporins was enforced, and in two subsequent periods: July to November 2010 and February to November 2011. The samplings were done at 11 slaughter plants covering 94% of the total number of pigs slaughtered in Denmark, as previously described. Sampling was weighted, meaning that the number of pigs sampled was proportional to the number of pigs slaughtered at each slaughterhouse.

Sampling of pigs at farm level

Five stool samples were collected in different pigsties (mainly with slaughter pigs) by veterinary control personnel during the regular control visits. The samples were mixed into one pooled sample and sent to the DVFA regional laboratories for analysis the same day. The farms were sampled once within the same year. The veterinary control personnel were asked to take samples in slaughter pigsties or, in cases where no slaughter pigs were present, another age group, preferably the oldest pigs in the herd.

Sampling of Danish retail pork

Samples from Danish pork were randomly collected from retail stores and outlets in all regions of Denmark as part of the Danish integrated surveillance programme (DANMAP), as previously described. The samples were collected from February to October in 2009, from January to December in 2010 and from January to October in 2011.

Isolation of ESC-producing E. coli and detection of ESC genes

Presumptive ESC-producing E. coli were isolated by adding 1 g of faeces or pooled stool sample to 10 mL of MacConkey broth (Oxoid CM5a) with 1 mg/L ceftriaxone (Sigma C5793–1G) followed by incubation for 16–18 h at 44°C. A 10 μL aliquot was streaked on MacConkey agar with 1 mg/L ceftriaxone and incubated overnight at 44°C, and a maximum of three colonies were subcultured. The same procedure was used for pork except that 5 g of meat was added to 45 mL of broth, E. coli was identified on CHROM Orientation agar (Becton Dickinson a/s). The ESC genotype was determined by the use of PCR, sequencing and a microtubine DNA array system as previously described. In brief, isolates from pigs were first screened for bla<sub>CTX-M</sub> genes by PCR and sequenced; if negative, the isolates were further screened for bla<sub>2</sub>, bla<sub>TEM</sub> and up-regulated ampC. Isolates negative for these ESC genotypes were further screened with a microtubine DNA array system (ClonDiag, Germany) and Identibac Amr-ve array tubes (New Haw, Addlestone, UK) and susceptibility was tested with discs (Oxoid, UK): cefotaxime (30 μg), ceftazidime (30 μg), cefotaxime (30 μg), cefotaxime + clavulanic acid (30 + 10 μg) and ceftazidime + clavulanic acid (30 + 10 μg); CLSI standards were followed. E. coli ATCC 25922 was used for quality control.

Statistical tests

Statistical significances of differences between proportions of samples positive for ESC-producing E. coli were calculated using the χ<sup>2</sup> test or Fisher's exact test (two-tailed) when the number of positive samples was low (<5). Estimation of exact 95% (two-sided) CIs for proportions was based on binomial probability distributions as previously described.

A simple linear regression analysis (using GraphPad Prism version 5) was performed in order to evaluate whether there were any significant trends in the dataset. A trend was determined as declining if the slope was negative and differed significantly from zero, increasing if the slope was positive and differed significantly from zero, or at a steady state if the slope did not differ significantly from zero (using GraphPad Prism version 5).

Results and discussion

The consumption of cephalosporins for pigs in each quarter is shown in Figure 1(a). The consumption of cephalosporins was highest before the ban was enforced, with the highest usage in the first quarter of 2009 (26.3 kg). Just before the ban was enforced 21.9 kg was used (second quarter of 2010). In the quarter when the ban was implemented (third quarter of 2010), usage decreased to 1.87 kg and in the following quarters 0.54–0.17 kg was used (Figure 1a).

The percentages of samples positive for ESC-producing E. coli from pigs at slaughter were 10.8% (85/786; 95% CI 8.73%–13.2%), 11.8% (48/407; 95% CI 8.82%–15.3%) and 3.6% (28/777; 95% CI 2.41%–5.17%) in 2009, 2010 and 2011, respectively. No statistically significant difference (P = 0.6, χ<sup>2</sup> test) in occurrence of samples positive for ESC-producing E. coli between 2009 and 2010 was observed, but between 2009 and 2011 a statistically significant decrease was observed (P < 0.001, χ<sup>2</sup> test) (Figure 1).

For pigs at farm level, 11% (11/99; 95% CI 5.7%–19%) (74 from slaughter pigsties) and 0% (0/78; 95% CI 0%–4.6%) (75 from slaughter pigsties) were positive for ESC-producing E. coli.
in 2010 and 2011, respectively. A statistically significant decrease was observed from 2010 to 2011 ($P=0.003$, Fisher’s exact test). Samples positive for ESC-producing E. coli were collected in the third [5% (1/20); 95% CI 0.13%–24.9%] and fourth quarter [15% (10/65); 95% CI 7.6%–26.5%] of 2010. Since the fraction of samples taken from pigsties with age groups other than slaughter pigs (mainly sows) was larger in 2010 than in 2011, the occurrence rates of ESC-producing E. coli from pooled samples taken exclusively from slaughter pigsties were compared. This also resulted in a significantly lower number of pooled samples positive for ESC-producing E. coli in 2011 [0% (0/75); 95% CI 0%–4.8%] compared with 2010 [8.1% (6/74); 95% CI 3.0%–16.8%] ($P=0.028$, Fisher’s exact test). In pork, the occurrence of ESC-producing E. coli was 1.9% (3/153; 95% CI 0.4%–5.6%), 1.1% (2/184; 95% CI 0.1%–3.9%) and 0.9% (2/225; 95% CI 0.1%–3.2%) in 2009, 2010 and 2011, respectively. The samples positive for ESC-producing E. coli were collected in the second [3% (2/74); 95% CI 0.3%–9.4%] and third quarter [3% (1/30); 95% CI 0.08%–17.2%] of 2009, in the second quarter [3% (2/70); 95% CI 0.4%–9.9%] of 2010, and in the second [1.5% (1/69); 95% CI 0.04%–7.8%] and third quarter [1.7% (1/58); 95% CI 0.04%–9.2%] of 2011. The lowest level was observed in 2011, although no significant differences between years were observed (Figure 1b).
In pigs the $\text{bla}_{\text{CTX-M-1}}$ gene was most often detected, accounting for 63% of the genes detected, but genes such as $\text{bla}_{\text{CTX-M-14}}$ and $\text{bla}_{\text{CTX-M-35}}$ were also detected (Figure 1b).

A simple linear regression was performed on the occurrence of ESC-producing $E. \text{coli}$ in pigs at slaughter each month before and after the intervention (Figure 1a and Table 51; available as Supplementary data at JAC Online). Prior to the intervention, the slope of the linear regression (0.01849 ± 0.3926) did not differ significantly from zero ($P = 0.96$), suggesting that the levels were at a steady state. After the intervention, the slope of the linear regression ($-0.6576 ± 0.2167$) was found to decline and differed significantly from zero ($P = 0.01$), suggesting that the intervention had a significant effect in reducing the number of ESC-producing $E. \text{coli}$. Figure 1(a) also shows the occurrence of ESC-producing $E. \text{coli}$ quarterly before and after the intervention, and a trend line based on a yearly moving average also shows a decline ~6 months after the intervention was enforced.

The genes encoding ESCs are often linked to other resistance genes on plasmids or other genetic elements, thereby mediating resistance to other, unrelated antimicrobial agents.\textsuperscript{10} Shortly after the voluntary cessation of all use of cephalosporins in pig production, the Danish government issued a ‘yellow card’ to the pig farmers who had the highest consumption per pig produced. This has led to an overall reduction in use for therapy of almost 25% during the last 2 years.\textsuperscript{11} Thus, it cannot be excluded that the reduced overall use has also been of importance; however, the most likely explanation for the major reduction in the occurrence of ESC-producing $E. \text{coli}$ among Danish pigs is the complete cessation of the use of cephalosporins. Together with other observations that major reductions in antimicrobial consumption can lead to major reductions in resistance,\textsuperscript{1,2} this has promise for future interventions for the benefit of human and animal health.

In conclusion, the discontinuation of an already low use of cephalosporins in pig production has significantly reduced the occurrence of ESC-producing $E. \text{coli}$.

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**References**


**Transparency declarations**

None to declare.

**Supplementary data**

Table 51 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).