Correlation between apramycin and gentamicin use in pigs and an increasing reservoir of gentamicin-resistant *Escherichia coli*

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**Objectives:** Resistance towards the veterinary drug apramycin can be caused by the *aac(3)-IV* gene, which also confers resistance towards the important human antibiotic gentamicin. The objectives of this study were to investigate the temporal occurrence and the genetic background of apramycin and gentamicin resistance in *Escherichia coli* strains from pork, healthy pigs and diagnostic submissions from pigs and to investigate potential relationships to the use of apramycin and gentamicin at farm and national levels.

**Methods:** Data on Danish *E. coli* isolates from healthy pigs (indicator bacteria), diagnostic submissions from pigs (clinical isolates) and pork were obtained from the national surveillance of antimicrobial resistance and from routine diagnostic laboratories. Antimicrobial consumption data were obtained from the Danish Medicines Agency (1997–2000) and from the VetStat database (2001–2004). The genetic background for gentamicin resistance was investigated by PCR. Relationships between antimicrobial usage and resistance were analysed by χ² test and logistic regression.

**Results:** At the farm level, the occurrence of apramycin/gentamicin cross-resistance was correlated to the use of apramycin (*P* < 0.001). At the national level, occurrence of apramycin/gentamicin cross-resistance in clinical *E. coli O149* isolates was significantly correlated with the amounts and duration of apramycin use. The *aac(3)-IV* gene was detected in all tested cross-resistant isolates.

**Conclusions:** Apramycin consumption at farm level is most probably driving the increasing occurrence of apramycin/gentamicin cross-resistant [*aac(3)-IV* positive] *E. coli* in diseased pigs and healthy finishers at slaughter. The duration of use and amounts used both had a significant effect on the prevalence of apramycin/gentamicin cross-resistance in diseased weaning pigs at the national level.

Keywords: aminoglycosides, resistance epidemiology, animal reservoirs, *E. coli*

**Introduction**

Gentamicin is classified as a critically important drug in human medicine.⁴ Gentamicin is a first-choice drug (in combination with β-lactams) for severe human infections (e.g. sepsis and endocarditis) in Danish Hospitals.⁵,⁶ Therefore, spread of gentamicin-resistant *Escherichia coli* strains to humans is of great concern.

Resistance to gentamicin and other aminoglycosides is usually transmissible, encoded on conjugative R-plasmids, and often linked to resistance to other antimicrobials.⁵,⁶ Genes encoding aminoglycoside-modifying enzymes that cause resistance to several aminoglycosides (cross-resistance) have been identified.⁷ The gene *aac(3)-IV* is the only identified gene causing enzymatic cross-resistance between gentamicin and apramycin.⁸ Apramycin resistance related to the *aac(3)-IV* gene was originally isolated in 1981 in France and the gene was only found in the animal reservoir.⁹ During the following years the gene spread rapidly in the animal reservoir in France, Belgium and Great Britain.
In 1986, aac(3)-IV was first detected in Enterobacteriaceae isolated from human patients.10

Gentamicin and apramycin were introduced into veterinary therapy in the early 1980s in several European countries.8 In Denmark, a gentamicin product was authorized in 1991 for oral use in piglets and has also been administered in extemporaneously prepared drugs.11 Several apramycin products were authorized for oral use in production animals in 1998.11

In Denmark, detailed information on aminoglycoside use in food-producing animals is registered in the Danish veterinary drug-monitoring programme, VetStat.12 The VetStat database contains data at farm level on consumption of all prescription drugs purchased by animal owners or used by veterinarians, including information on animal species, age group, disease group and farm identity.

In Danish pig production, gentamicin and apramycin are widely used, as described in this paper. Resistant E. coli are commonly isolated from diseased pigs,13 and E. coli from pigs may be an important reservoir for transfer of gentamicin resistance genes or bacteria to humans.14

The objectives of this study were: (i) to investigate temporal association between the use of gentamicin and apramycin and the occurrence of gentamicin and apramycin resistance in E. coli isolates from pigs in Denmark; (ii) to investigate whether isolates were cross-resistant to gentamicin and apramycin; and (iii) to investigate whether the responsible genes were encoded by the aac(3)-II, aac(3)-IV or ant(2′)-I gentamicin resistance genes.

Methods

Aminoglycoside usage data

National-level data on the total antimicrobial consumption (kg of active compound) in production animals during 1997–2000 were derived from the Danish Medicines Agency, based on data from pharmaceutical companies and feed mills. These data did not cover extemporaneously prepared drugs. For 2001–2004, detailed data on antimicrobial use by animal species were derived from the Danish veterinary drug-monitoring programme, VetStat.12 In this study, apramycin and gentamicin consumption was measured in defined animal daily doses for a 15 kg pig (ADD kg -1 per 1000 pigs produced disregarding target age groups, because these aminoglycosides are usually used in weaners.13,15 Antimicrobial use at the national level was adjusted for variation in size of population ‘at risk’ in comparison with the total number of pigs slaughtered annually. Where farm identity of origin for the indicator E. coli and clinical isolates of E. coli O149 was available, data on apramycin and gentamicin consumption within 1 year before sampling were extracted at farm level, including usage on multisite facilities. Because antimicrobial consumption data were not available at farm level before 2001, data could only be extracted for farms from which E. coli strains were isolated during 2002–2004.

Bacterial isolates

E. coli was isolated from samples from healthy pigs at slaughter (indicator isolates), diagnostic submissions from pigs (clinical isolates) and pork.

Indicator bacteria isolates

Samples of caecal content were collected at slaughter by company staff or meat inspection staff at slaughter plants for the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP).13 The pig slaughter plants included in DANMAP process 95% of the pigs slaughtered in Denmark. The number of samples obtained at each plant was proportional to the annual number of pigs slaughtered. Each sample represented one farm.

Clinical isolates

All isolates were derived from diagnostic submissions (nationwide coverage) to The Danish Institute of Food and Veterinary Research (DFVF) or the Danish Bacon and Meat Council (DBMC). The material was gut contents or faeces from cases of diarrhoea, predominantly from weaning pigs. When haemolytic E. coli was isolated or when E. coli was the predominant bacterial agent present in culture, the isolates were serotyped. Enterotoxigenic E. coli (ETEC) is an important pathogen, causing diarrhoea in newborn and post-weaning pigs, with serotypes O8, O45, O138, O139, O141, O147, O149 and O157 being the most common pathogenic strains in weaning pigs in Denmark.16 Serotype testing at the routine diagnostic laboratory at DFVF comprised these serotypes in addition to O64. DANMAP comprises part of the E. coli O149 from DFVF and DBMC. Serotype O149 is used to follow the trend in resistance occurrence because it is the predominant serotype, universally associated with diarrhoea in neonatal and weaning pigs.16 In this study, all O149 isolates from the DBMC and all E. coli O149 from DFVF from 1998 to 2004 were included. Additionally, other types and non-typed strains isolated at DFVF during 2003–2004 were included for comparison.

Pork samples were collected at wholesale and retail outlets by the regional food control authorities on request specifically for DANMAP. The isolates were taken both from imported (<10%) and Danish (>90%) meat.

Isolation methods used were described in the DANMAP reports,13 for the pork and indicator isolates, and, in a previous study, for the clinical samples.8

Susceptibility testing

Susceptibility to gentamicin and apramycin was determined at DFVF by the microbroth dilution method using the Sensititre antimicrobial susceptibility system according to the manufacturer (Trek Diagnostics Systems Ltd, East Grinstead, UK). E. coli ATCC 25922 was used for quality control. The wells were inoculated and incubated according to the National Committee for Clinical Laboratory Standards (in 1997–2003) and Clinical and Laboratory Standards Institute guidelines (in 2004). The breakpoints used for gentamicin and apramycin were 28 and 32 mg/L, respectively, in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards.13

Identification of resistance genes

At the National Center for Antimicrobials and Infection Control, PCR was used to screen for three genes, encoding aminoglycoside-modifying enzymes, including ant(2′)-I, aac(3)-II and aac(3)-IV. The primers published by Sandvang and Aarestrup17 were used: aac(3)-IV-F, 5′-TCA AAG TGG TCC TGG TCC ACA GC-3′; aac(3)-IV-B, 5′-AGT TGA CCC AGG GCT GTC GC-3′; ant(2′)-I-F, 5′-GGG CGC GTC ATG GAG GAG TT-3′ and ant(2′)-I-B, 5′-TAT CGC GAC CTG AAA GGC GC-3′. Klebsiella pneumoniae 23823 [aac(3)-II], Salmonella Typhimurium DT170 0304 M74945 [aac(3)-IV] and E. coli L58058 [ant(2′)-I] were used as positive controls. E. coli MT102 and E. coli K12 362 were used as negative controls. Identification of resistance genes was performed on 36 isolates, comprising
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9 indicator strains (three from 2003 and six from 2004) and 25 apramycin/gentamicin cross-resistant and 2 gentamicin-resistant clinical strains (four, six, three, five and nine isolates from the years 2000–2004, respectively).

**Statistical methods**

Within each sample type (slaughter house samples, clinical submissions and pork), the proportion of apramycin- or gentamicin-resistant isolates was calculated for each year. The effect of yearly apramycin consumption in pigs at the national level and the effect of time (year) since the introduction of apramycin on the prevalence in clinical isolates was analysed by logistic regression (SAS® EG version 3.0), and 95% likelihood confidence intervals (CI) were calculated for the odds ratio (OR). Pearson $\chi^2$ and deviance criteria were used for assessing goodness of fit. The $\chi^2$ test was used to test for differences in prevalence of resistance or differences in resistance pattern (occurrence of apramycin resistance, gentamicin resistance and apramycin/gentamicin cross-resistance) between the three groups of clinical *E. coli* isolates (O149, other types, non-typeable isolates). The $\chi^2$ test with Yates’ continuity correction was used to test for differences in apramycin and gentamicin use between farms from which the susceptible and resistant clinical strains originated.

**Results**

**Antimicrobial consumption**

In Denmark, the total meat production increased by 1.6% from 1999 to 2001 while the annual consumption of aminoglycosides in animals increased by 48% from 6.4 to 9.5 tonnes, primarily due to increasing use of oral preparations of neomycin and apramycin. Apramycin is used almost entirely in pig production with only a minor part used in calves. After its marketing in 1998, consumption of apramycin in pigs increased from 0 to 767 kg in 2001, and gradually decreased to 411 kg in 2004, while the production of pigs increased from 22.7 million in 1998 to 23.2 million in 2001 and to 25.1 million pigs in 2004. For pigs, apramycin was primarily (>88% in 2004) used in weaners (7.5–30 kg pigs), less in sow herds for piglets (>88% in 2004) and rarely in finishers. In 2004, the consumption of apramycin was 2.4 ADD$_{15}$/1000 finishers produced, as compared with 54 ADD$_{15}$/1000 weaners produced, including weaners produced for export.

The consumption of gentamicin in authorized medicinal products was low (2.5–3 kg annually) during 1999–2004. Production figures from the industry indicated an estimated 30 kg veterinary consumption of gentamicin in 1999 (E. Jacobsen, the VetStat programme, the Danish Institute for Food and Veterinary research, personal communication), but the use of gentamicin in extemporaneously prepared drugs before 2001 was otherwise not known. The consumption of gentamicin probably decreased after the authorization of apramycin products, because use of extemporaneously prepared drugs is not legal when an alternative authorized medicinal product (in this case apramycin) is available. In 2001, an estimated 50 kg of gentamicin was used in pigs, and 75% of the gentamicin consumption in pigs was used in weaners. The consumption decreased to about 2 kg annually in 2003–2004, when gentamicin was almost entirely used in a product formulated for piglets.

**Bacterial isolations and antimicrobial susceptibility**

During 1997–2004, 2109 indicator *E. coli* and 1318 clinical isolates of *E. coli* O149 were isolated from pigs and tested for apramycin and gentamicin resistance. During 1999–2004, 560 *E. coli* strains were isolated from pork samples (of which >90% were of Danish origin). The annual number of samples and findings of resistance are shown in Table 1. Apramycin- or gentamicin-resistant *E. coli* isolates were not found in any of the pork samples from 1999 to 2004. During 1997–2001, neither apramycin nor gentamicin resistance was found in indicator *E. coli* (0/1294 isolates). In 2002, one (0.3%) gentamicin-resistant indicator *E. coli* strain was isolated. In 2003 and 2004, three (0.9%) and seven (3.4%) resistant indicator apramycin/gentamicin cross-resistant *E. coli* were isolated, respectively. Regarding clinical isolates of serotype O149, the annual number of isolates resistant to gentamicin (not apramycin) has remained at a low level, 13/1214 isolates from 1998 to 2004 (Table 1). In 1997 and 1998, apramycin resistance was not found in clinical isolates (0/222 isolates). During the years 2000–2004, 2.6%, 9.7%, 15.3%, 10.9% and 14.6%, respectively, of the *E. coli* O149 were apramycin/gentamicin cross-resistant.

**Table 1.** Apramycin (APR) and gentamicin (GEN) resistance in isolates of *E. coli* from pigs

<table>
<thead>
<tr>
<th>Year</th>
<th>Isolates from pork, n</th>
<th>no. of resistant isolates (%)</th>
<th>Clinical isolates (E. coli O149)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>GEN</td>
<td>GEN + APR</td>
</tr>
<tr>
<td>1997</td>
<td>–</td>
<td>106</td>
<td>0</td>
</tr>
<tr>
<td>1998</td>
<td>–</td>
<td>298</td>
<td>0</td>
</tr>
<tr>
<td>1999</td>
<td>80</td>
<td>284</td>
<td>0</td>
</tr>
<tr>
<td>2000</td>
<td>62</td>
<td>299</td>
<td>0</td>
</tr>
<tr>
<td>2001</td>
<td>48</td>
<td>304</td>
<td>0</td>
</tr>
<tr>
<td>2002</td>
<td>69</td>
<td>293</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>2003</td>
<td>123</td>
<td>317</td>
<td>0</td>
</tr>
<tr>
<td>2004</td>
<td>178</td>
<td>208</td>
<td>0</td>
</tr>
</tbody>
</table>

*a*Not analysed.
In clinical *E. coli* isolates other than serotype O149 and non-typed isolates from the routine diagnostic laboratory at DFVF, gentamicin or apramycin resistance was found in 61 of 996 isolates during 2003–2004 (Table 2). The occurrence of apramycin/gentamicin cross-resistance was not significantly different in serotype O138 and O149 but significantly lower in other serotypes (χ² = 13.4, df = 1, P < 0.001) and non-type-able isolates (χ² = 21.8, df = 1, P < 0.001) than in the *E. coli* O149 isolated in 2003 and 2004.

**Antimicrobial consumption and susceptibility**

In Figure 1, the prevalence of resistance in porcine clinical isolates (*E. coli* O149) and indicator *E. coli* was compared with the use of apramycin and gentamicin in pig production. Logistic regression showed that both time since approval of apramycin (*P* < 0.001) and the yearly amount of apramycin consumption (*P* = 0.009) were significant, while the interaction between these variables was non-significant. The odds ratios were OR = 1.6 (95% CI: 1.1–2.3) for a 50 U change in the yearly consumption of apramycin (1 U = ADD₁₅kg/1000 pigs produced) and OR = 1.9 (95% CI: 1.5–2.6) for time (year) after approval of apramycin.

At farm level, apramycin had been used in 28 of 46 farms within 1 year before isolation of apramycin/gentamicin-resistant *E. coli* O149 from the respective farms. Gentamicin had been used in seven of the 28 farms for treatment of piglets, in very small amounts compared with apramycin use. Gentamicin had been used in two additional farms and neither gentamicin nor apramycin had been prescribed for the remaining 16 farms. Regarding the susceptible *E. coli* O149 from 2003 to 2004, farm identity was known for 256 farms. Within these farms, apramycin had been used on 53 farms, and gentamicin had been used on 30 farms within 1 year before the susceptible isolates were obtained. The use of apramycin was significantly (Yates χ² = 30, *P* < 0.001; OR = 6.0) higher in farms where cross-resistant isolates were found, while use of gentamicin was not significantly different (Yates χ² = 1.5, *P* = 0.22, OR = 1.8) in farms from which susceptible and resistant isolates originated.

Regarding resistant indicator *E. coli*, neither apramycin nor gentamicin had been prescribed for finishers in the respective farms within the past year before isolation. Five of the ten apramycin/gentamicin-resistant indicator isolates originated from farms in which apramycin had been prescribed for weaners (four farms) and/or sow herds (three farms) within 1 year before isolation. In one of these farms and in another farm, gentamicin had been prescribed for piglets. Within the remaining four farms, two did not house weaners or sows, and apramycin or gentamicin may have been used for the pigs before they were moved to the finisher farms.

Farm identity was known for the 456 apramycin/gentamicin-susceptible indicator isolates from 2003 to 2004. Within the 456 farms, apramycin had been used in 36 farms, and gentamicin had been used for weaners on 10 farms within the past year before

<p>| Table 2. Apramycin (APR) and gentamicin (GEN) resistance in clinical isolates of different <em>E. coli</em> types (DANMAP and DFVF isolates, 2003–2004) |</p>
<table>
<thead>
<tr>
<th>Serotype</th>
<th>APR</th>
<th>GEN/ APR</th>
<th>GEN</th>
<th>Susceptible</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>O149</td>
<td>3 (1.9)</td>
<td>39 (19.7)</td>
<td>5 (3.8)</td>
<td>258 (279.7)</td>
<td>305</td>
</tr>
<tr>
<td>O8</td>
<td>1 (0.4)</td>
<td>0 (4.2)</td>
<td>0 (0.8)</td>
<td>64 (59.6)</td>
<td>65</td>
</tr>
<tr>
<td>O45</td>
<td>0 (0.1)</td>
<td>2 (1.7)</td>
<td>0 (0.3)</td>
<td>25 (25.1)</td>
<td>27</td>
</tr>
<tr>
<td>O138</td>
<td>2 (0.3)</td>
<td>5 (3.4)</td>
<td>2 (0.6)</td>
<td>43 (47.7)</td>
<td>52</td>
</tr>
<tr>
<td>O139</td>
<td>0 (0.4)</td>
<td>1 (3.9)</td>
<td>1 (0.7)</td>
<td>58 (55.0)</td>
<td>60</td>
</tr>
<tr>
<td>O141</td>
<td>0 (0.1)</td>
<td>1 (1.1)</td>
<td>0 (0.2)</td>
<td>16 (15.6)</td>
<td>17</td>
</tr>
<tr>
<td>Other types&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 (0.1)</td>
<td>0 (1.4)</td>
<td>0 (0.3)</td>
<td>21 (19.3)</td>
<td>21</td>
</tr>
<tr>
<td>Haemolytic nt&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2 (1.6)</td>
<td>13 (17.0)</td>
<td>3 (3.2)</td>
<td>246 (242.1)</td>
<td>264</td>
</tr>
<tr>
<td>Non-haemolytic nt&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0 (3.0)</td>
<td>23 (31.6)</td>
<td>5 (6.0)</td>
<td>462 (449.3)</td>
<td>490</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>84</td>
<td>16</td>
<td>1193</td>
<td>1301</td>
</tr>
</tbody>
</table>

<sup>a</sup>All had intermediate susceptibility to gentamicin (MIC = 4 mg/L).

<sup>b</sup>Expected values (in parentheses) according to the null hypothesis (random distribution of resistance genes).

<sup>c</sup>Serotypes O64, O147 and O157.

<sup>d</sup>Non-type-able strain using the panel of serotypes mentioned.

**Figure 1.** Consumption of apramycin and gentamicin in pigs and occurrence of resistance in porcine isolates of *E. coli*, Denmark 1999–2004. ‘a’ indicates that consumption of gentamicin in pigs before 2001 was unknown. ‘b’ represents ADD₁₅ = daily dose for a 15 kg pig. GEN = gentamicin resistant (including apramycin cross-resistant isolates). APR = apramycin resistant.
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isolation at slaughter. The use of apramycin was significantly higher in weaners in the farms from which the apramycin/gentamicin-resistant indicator *E. coli* originated ($\chi^2 = 14.5$; $P = 0.0001$; OR = 12).

**Detection of gentamicin resistance genes**

The aac(3)-IV gene was identified in all tested apramycin/gentamicin cross-resistant isolates, including 25 clinical isolates of *E. coli* (20 of these were serotype O149) and nine indicator *E. coli*. None of the three selected resistance genes was present in two gentamicin-resistant but apramycin-susceptible clinical isolates of *E. coli* O149.

**Discussion**

Aminoglycosides are widely used in animal production as compared with the human population, suggesting that the animal reservoir may be important to the occurrence of resistance in humans. In 2004, a total of 31 kg of aminoglycosides was used in the Danish population of 5 million inhabitants while 370 kg of apramycin and 4 kg of gentamicin was used therapeutically for a population of approx. 10 million pigs in Denmark.\(^{13,18}\) The nationwide detailed data of veterinary antimicrobial use in VetStat and surveillance data on occurrence of resistance collected in DANMAP makes it possible to correlate information on antimicrobial consumption patterns at animal species level with the occurrence of resistance at different levels in the animal-human food chain.

In Denmark, apramycin resistance in porcine *E. coli* was rare before the approval of apramycin.\(^{17}\) Regarding *E. coli* O149 from clinical submissions, apramycin/gentamicin cross-resistance was identified in only one strain in 1995–1996\(^{17}\) and none in 1997–1998. In 2000, 2 years after the approval of apramycin for veterinary use in Denmark, apramycin resistance in *E. coli* from pigs was found in 11 (2.9%) clinical isolates. Ten of these isolates were also resistant to gentamicin. The use of apramycin increased markedly until 2001 and decreased in the following years, while the occurrence of apramycin/gentamicin cross-resistance continued to increase until 2002 and remained unchanged in 2003 and 2004. At the national level, time since approval of apramycin and amount of apramycin consumed yearly had a significant effect on apramycin/gentamicin cross-resistance among *E. coli* O149. A gradual increase in resistance was observed, from none in 1998 to 15% in 2002. Results at the farm level show a significant correlation between apramycin use within 1 year prior to sampling and occurrence of apramycin/gentamicin cross-resistance. The odds of apramycin use were six times higher when resistant clinical isolates were found, as compared with susceptible clinical isolates. Also, the finding of resistant indicator *E. coli* at slaughter was significantly related to use of apramycin. When a resistant indicator *E. coli* isolate was detected, the odds that the weaners had been treated with apramycin were 12 times higher than if a susceptible isolate was detected. Gentamicin, which is primarily used in piglets, did not have any significant effect on the occurrence of apramycin-gentamicin cross-resistance in weaners or at slaughter.

In 2001, the first apramycin/gentamicin-resistant isolates of indicator *E. coli* were found. Apramycin and gentamicin resistance in indicator *E. coli* has remained at a low level with an increasing trend (non-significant) during 2002–2004. In 2004, apramycin/gentamicin cross-resistance was exhibited by 3.4% of the indicator bacteria. Apramycin or gentamicin resistance was not detected in *E. coli* isolated from pork. However, due to the low sensitivity of the detection method of resistant *E. coli* (using non-selective media for isolation), the presence of resistant *E. coli* may be more frequent in pork than indicated by this study.\(^{19}\)

The main indication for apramycin is treatment of *E. coli* diarrhoea in piglet nurseries and post-weaning medication.\(^{20–23}\) In Denmark, apramycin was used in 1% (54 ADD15/1000 pigs) of the antimicrobial treatments of diarrhoea in weaning pigs in 2004 (total gastrointestinal treatment was 78% of 7100 ADD15/1000 pigs\(^{13}\)), but the percentage is probably higher for the main indication, *E. coli* diarrhoea. A study by Mathew et al.\(^{23}\) indicated that other young pigs within a farm may be a probable source of resistance, with transfer as early as 7 days of age, and the percentage of isolates resistant to apramycin/gentamicin increased rapidly from 7 days to 35 days of age, most probably due to the increased drug use at time of weaning. Boerlin et al.\(^{24}\) demonstrated that antimicrobial resistance epidemiology differs significantly between pathogenic strains (ETEC) from weaners and commensal *E. coli* from healthy finishers. The load of resistant *E. coli*, in particular the pathogenic strains causing post-weaning diarrhoea, is likely to decay after moving the pigs to the finisher farms, where apramycin is rarely used (2.4 ADD15 = 0.67ADD15/1000 finishers produced), explaining the significantly lower level of resistance in *E. coli* at slaughter. The ETEC serotypes causing diarrhoea in weaning pigs are different from the commensal serotypes dominating in older pigs.\(^{25,26}\) Thus, occurrence of apramycin/gentamicin resistance in pigs at slaughter may be due to transfer of resistance genes from pathogenic strains to commensal strains and/or persistence of resistant commensal strains from the weaning herds into the finisher herds. The persistence of the resistant strains may be due to co-selection by other drugs used in finishers. This hypothesis was supported by the present findings, that apramycin had not been used in finishers on the farms from which the apramycin/gentamicin-resistant indicator *E. coli* originated, while usage in the young pigs was significantly higher compared with the farms where the susceptible indicator isolates originated. High frequencies of resistance to tetracycline, sulphonamides, streptomycin and spectinomycin in clinical *E. coli* isolates have been reported from several countries.\(^{13,27–29}\) In 2004, the prevalence of resistance in the DANMAP clinical isolates (49 of the *E. coli* O149 used in this study) was 86%, 76%, 67% and 59% for the four antimicrobials respectively and these types of resistance were accordingly frequent in the apramycin/gentamicin-resistant isolates. Furthermore, associations between tet(A), sul1, aadA and aac(3)-IV have been observed in *E. coli* obtained from Canadian pigs.\(^{24}\) The prevalence of resistance in indicator strains was markedly lower than in clinical strains. In 2004, the prevalence of resistance to tetracycline, sulphonamides, streptomycin and spectinomycin was 44%, 47%, 48% and 34%, respectively, and 47% of the isolates were multiresistant (≥4 antimicrobials) while 27% of the isolates were susceptible to all 17 antimicrobials tested (data collected for DANMAP\(^{13}\)). In our study, all 10 apramycin/gentamicin-resistant indicator *E. coli* isolates were multiresistant (data from DANMAP\(^{25}\)). Eight isolates were resistant to ≥6 antimicrobials (including gentamicin and apramycin). Eight of the isolates were resistant to tetracycline, eight were resistant to sulphonamide and nine isolates were resistant
to streptomycin. Additionally, resistance to trimethoprim (5), neomycin (4), spectinomycin (3), chloramphenicol (2) and nalidixic acid (1) occurred. These findings support the hypothesis that co-selection might affect the persistence of apramycin/gentamicin-resistant isolates in finishers.

Regarding the clinical isolates, Boerlin et al. found a higher frequency of apramycin resistance among the ETEC strains as compared with non-ETEC strains from diseased animals. In our study, occurrence of resistance in the porcine pathogenic strains, E. coli O149 and O138, was higher than in other strains isolated from clinical samples from pigs. These strains were the most frequent pathogenic strains (ETEC) accounting for 64.8% of the isolates from weaning diarrhoea in a previous Danish study. The higher occurrence of resistance in these strains remains unexplained, although the strains are more prone to be exposed to apramycin, because these strains are most frequently involved in weaning diarrhoea.

The aac(3)-IV gene was found in all apramycin/gentamicin cross-resistant isolates tested, suggesting that it is the predominant gene responsible for this resistance pattern in Danish pigs. In contrast, a previous Danish study showed a higher prevalence of ant(2')-I and aac(3)-IIa as compared with aac(3)-IV in gentamicin-resistant E. coli obtained from pigs at the time (1995–1996). However, in both studies, aac(3)-IV was found in all apramycin-resistant strains, in accordance with findings in the USA, where >90% of the apramycin-resistant isolates were carrying the aac(3)-IV gene.30,31 The prevalence of isolates resistant to gentamicin and susceptible to apramycin has remained low in clinical samples and was not found in indicator E. coli at slaughter or from food samples.

Gentamicin is used for systemic treatment of critical human infectious diseases, such as bacteraemia, in hospitals. E. coli bacteraemia is secondary to several primary infections of which urinary tract infections are among the most common. A recent study has demonstrated a close resemblance between certain food-borne and human isolates of extraintestinal pathogenic E. coli, suggesting a possible transmission of E. coli causing urinary tract infections through food products. Due to the risk of transfer of gentamicin resistance genes or gentamicin-resistant E. coli from animals to humans and the consequent risk of difficulty in treating infections with gentamicin-resistant E. coli, the presence of gentamicin-resistant E. coli in animals is of great concern.

In a Danish study, only the aac(3)-IV gene was detected in E. coli isolates from wastewater from a residential area, when screening for the presence of ant(2')-I, aac(3)-II and aac(3)-IV, which are among the most prevalent gentamicin resistance genes in human E. coli. However, the yearly consumption of gentamicin for human use by general practitioners in Denmark is close to zero (3 DDD/year per million inhabitants in 2004). In hospital wastewater, the ant(2')-I and aac(3)-II genes were more frequent than aac(3)-IV. Thus, their study suggested a reservoir of aac(3)-IV genes possibly driven by some source other than the human gentamicin consumption. The apramycin/gentamicin cross-resistant E. coli in wastewater from the normal population may originate from the animal reservoir.

Based on our study, it is concluded that apramycin consumption is most probably driving the increasing occurrence of apramycin/gentamicin cross-resistance in pigs. The yearly amounts used and time since apramycin approval had a significant effect on the prevalence of occurrence in diseased weaning pigs at the national level. There might be a trend towards an increase in apramycin resistance in isolates from finishers at slaughter, which seems to be delayed compared with observed increase in apramycin resistance in E. coli from diseased pigs. The occurrence of multiresistance gives rise to concern that the occurrence of apramycin/gentamicin cross-resistance in finishers at slaughter may increase further, despite decreasing levels of apramycin and gentamicin consumption. The increasing occurrence of resistance to apramycin with cross-resistance to gentamicin in animals is of concern and should be under close surveillance. Resistance to apramycin and gentamicin in Enterobacteriaceae and other enteric pathogens generally remains low in pigs at slaughter and in food at retail, but the resistance may be present in pork below the detection level in this study. Several studies from Great Britain have found that ~26% of the gentamicin-resistant pathogenic E. coli strains from humans were carrying the aac(3)-IV gene.

The present study suggests that the occurrence of the aac(3)-IV gene in diseased and healthy pigs may be an increasing problem, which pleads for the prudent use of antimicrobials in pigs, considering the human health risk associated with apramycin/gentamicin cross-resistance.

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Transparency declarations
None to declare.

References
Apramycin and gentamicin resistance in *E. coli*


