Effect of the growth promoter avilamycin on emergence and persistence of antimicrobial resistance in enteric bacteria in the pig

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ABSTRACT


Aim: To assess the effect of the growth promoter avilamycin on emergence and persistence of resistance in enteric bacteria in the pig.

Methods and Results: Pigs (treated with avilamycin for 3 months and controls) were challenged with multi-resistant Salmonella Typhimurium DT104 and faecal counts were performed for enterococci, Escherichia coli, S. Typhimurium and Campylobacter (before, during and 5 weeks post-treatment). Representative isolates were tested for antibiotic resistance and for the presence of resistance genes. Avilamycin-resistant Enterococci faecalis (speciated by PCR) were isolated from the treated pigs and continued to be detected for the first week after treatment had ceased. The avilamycin-resistance gene was characterized by PCR as the emtA gene and speciation by PCR. MIC profiling confirmed that more than one strain of Ent. faecalis carried this gene. There was no evidence of increased antimicrobial resistance in the E. coli, Salmonella and Campylobacter populations, although there was a higher incidence of tetB positive E. coli in the treated pigs than the controls.

Conclusion: Although avilamycin selects for resistance in the native enterococci population of the pig, no resistant isolates were detected beyond 1 week post-treatment. This suggests that resistant isolates were unable to persist once selective pressure was removed and were out-competed by the sensitive microflora.

Significance and Impact of the Study: Our data suggest the risk of resistant isolates becoming carcass contaminants and infecting humans could be minimized by introducing a withdrawal period after using avilamycin and prior to slaughter.

Keywords: antimicrobial resistance, growth promoters, zoonotics.

INTRODUCTION

In the European Union, a third of antimicrobials used for veterinary purposes are administered as antimicrobial growth promoters (AGP) (Ungemach 2000). These have beneficial effects as animals grow faster and more uniformly, have a lower feed to weight-gain ratio and release less nitrogen into the environment (Corpet 2000). However, links between the use of AGPs and the emergence of resistance in enterococci and other Gram-positive bacteria have been reported for avoparcin (Bager et al. 1997; Wegener et al. 1997; Robredo et al. 2000), avilamycin, macrolides and bacitracin (Aarestrup et al. 2000b), and streptogramins (Aarestrup et al. 2000b; Robredo et al. 2000; Gambarotto et al. 2001).

Transmission of glycopeptide-resistant enterococci from food animals exposed to avoparcin, to humans is now well documented (Goosens 1998; MAFF 1998) and is having a
direct effect on human medicine. Indirect effects have also been experienced in the search for new antibiotics to target multi-resistant enterococci with for example the recent development of a new agent, evernimicin, which is structurally very similar to avilamycin. Investigations showed that avilamycin-resistant enterococci isolated from pigs and broilers already had a decreased susceptibility to evernimicin (Aarestrup 1998; Kropec et al. 2001; Aarestrup and McNicholas 2002).

In an attempt to address concerns that antimicrobial resistance selected for in food animals could be transmitted to humans, the European Union banned the use of avoparcin in 1997, and bacitracin, spiramycin, tylosin and virginiamycin in 1999. By 2006, a ban on all remaining growth promoters will be imposed. Since the bans, there has been a decrease in antibiotic-resistance genes in animal and human enterococci (indicator species) (MAFF 1998; van den Bogaard et al. 2000). However, there has been an increase in the use of therapeutic antimicrobials to treat food animals, and an increase in tetracycline and sulphonamide-resistant S. Typhimurium (Danish Integrated Antimicrobial Resistance Monitoring and Research Programme 2002) (Veterinary Medicines Directorate, 2002, UK; http://www.vmd.gov.uk), and tetracycline and fluoroquinolone resistant human Campylobacter isolates (Ministry of Food 2001). These observations indicate that growth promoters could have significant prophylactic properties and suggest that the benefits of the widespread ban of growth promoters may need to be more carefully weighed against adverse consequences.

The impact of the use of antimicrobials in food animals on human medicine could be minimized by reducing the potential for resistant enteric bacteria, selected during treatment, to move up the food chain. Therefore, the effect of avilamycin on the emergence and persistence of resistance in the native bacteria in the pig was investigated in an attempt to identify control strategies to minimize movement of resistance up the food chain. The concentrations of avilamycin A and avilamycin B (the two main components of MAXUS; commercial product name) excreted in the pig faeces, were also determined by HPLC. The level of AGP in faeces reflects the concentration to which the enteric bacteria are exposed and indicates the levels released into the environment, which could potentially be re-ingested by the pigs.

**MATERIALS AND METHODS**

**Animals**

Fourteen piglets, from three litters of Saddleback–Duroc cross and weaned at the age of 3 weeks, were housed as a single group for 2 weeks to allow the gut microflora to establish. They were then divided randomly into one group of eight and one group of six in two pens with individual HEPA filtration and fed a standard organic feed (Organic feed company, grower/finisher slurry) ad libitum for the duration of the experiment (4 months).

Each pig was screened for the presence of Campylobacter and Salmonella by plating out a 100 μl aliquot of 1 g of faeces emulsified in 9 ml phosphate-buffered saline (PBS) onto modified charcoal cefoperazone desoxycholate agar (CCDA, Oxoid) with selective supplement (CM739B, Oxoid) and a 500 μl aliquot of the same suspension onto brilliant green agar (BGA, Oxoid).

All procedures complied with Animals (Scientific Procedures) Act 1986 and were performed under Home Office Licence.

**Inoculation of S. Typhimurium DT104**

In order to investigate the effect of avilamycin on the zoonotic S. Typhimurium DT104, such as changes in phenotype or colonization, the pigs were artificially inoculated with three S. Typhimurium DT104 strains (S4668/98, S7660/96, S7652/96). Three strains were used to maximize the chances of at least one colonizing the pigs for the duration of the study. These were obtained from the culture collection at the Veterinary Laboratories Agency (VLA), Weybridge. The strains were isolated in 1996 and 1998 from apparently healthy pigs and were stored on dorset-egg slopes. All three strains had an identical phenotype and were resistant to ampicillin, tetracycline, chloramphenicol, sulphonamide and streptomycin. In addition, they were naturally resistant to furazolidone with an MIC of 128 mg l⁻¹, which was used as a selective marker for recovery from the background microflora. All 14 pigs were dosed with 1·7 × 10⁹ CFU of each S. Typhimurium DT104 strain (Delsol et al. 2003).

**Antibiotic treatment and sample collection**

Twenty-four hours after inoculating S. Typhimurium DT104 into the pigs, one group (n = 8) was exposed to feed supplemented with avilamycin (Maxus G200, Elanco: 0·1 kg ton⁻¹ of feed) for 3 months, whilst the second group (n = 6) acted as the control group and remained untreated. Avilamycin was administered via the feed as recommended by the manufacturer.

**Collection of faecal samples**

Faecal samples were collected by digital manipulation 24 h after the pigs were infected with S. Typhimurium DT104 and on days 3, 12, 18, 33, 47, 64 and 82 during treatment, and then on days 3, 15 and 34 post-treatment. Faeces (1 g) from each pig were emulsified in 9 ml PBS and 10-fold serial dilutions were made.
Isolation and identification of bacteria

Enterococci were isolated by spreading 500, 100 and 20 μl of the neat PBS suspension and 20 μl aliquots of the 10⁻¹ to 10⁻³ dilutions onto SB (Slanetz and Bartley) and grown at 42°C. Counts below the detection limit of 20 CFU were counted as 0 CFU g⁻¹ faeces. Purple colonies were counted as presumptive enterococci. Eight isolates per plate were then speciated by PCR as previously described by Dutka-Malen et al. (1995).

Prior to avilamycin treatment and once 2 weeks post-treatment, all pigs were screened for the presence enterococci MIC resistant to at least 4 mg l⁻¹ avilamycin by plating out 500 μl of neat PBS onto SB supplemented with 4 mg l⁻¹ (detection limit: 20 CFU g⁻¹ faeces).

The commensal E. coli population was isolated by spreading 20 μl aliquots of 10⁻¹ to the 10⁻⁴ faecal dilutions on MacConkey plates (Oxoid). The identity of representative isolates (n = 423; 8 CFU per plate) was confirmed by colony morphology on BGA and Rambach media (Oxoid).

To re-isolate the inoculated S. Typhimurium DT104, triplicates of 10, 100 and 500 μl aliquots of the PBS suspension were spread onto plates of MacConkey agar (Oxoid) supplemented with 32 mg l⁻¹ furazolidone (Sigma). Nonlactose fermenting colonies were enumerated and eight colonies per plate were confirmed as Salmonella by subsequent colony morphology on BGA and Rambach media (Oxoid).

Campylobacter was isolated on CCDA with selective supplement (CM739B, Oxoid). A 10-fold dilution series of the faecal suspension was prepared, and 20 μl aliquots of 10⁻¹, 10⁻³ and 10⁻⁵ dilution were spread onto half plates of CCDA and incubated in microaerophilic conditions at 37°C (5% O₂, 10% CO₂, 85% N₂). Eight colonies per plate were confirmed as Campylobacter by morphology, motility, and lack of growth in aerobic conditions at 20°C. Isolates were speciated by the biochemical tests: hippurate, indoxyl acetate and urease (Skirrow 1990).

Statistical analysis

Two-samples of equal variance t-tests were run separately on counts from the two groups of pigs before treatment and from counts before and 3 days post-treatment in the treated pigs.

Determination of susceptibility to antibiotics and cyclohexane

The MIC values for enterococci, E. coli and Salmonella were determined by an agar doubling dilution method following standard VLA protocols (Davies and Roberts 1999). Briefly, cultures grown overnight at 37°C in nutrient broth were diluted one-tenth in normal saline and inoculated, using a multipoint inoculator onto Isosensitest agar (Oxoid CM471) supplemented with dilutions of the antibiotics. Plates were incubated overnight at 37°C. Cyclohexane resistance was determined as previously described by Randall et al. (2001). Enterococci NCTC 775, NCTC 7171, NCTC 12202 and E. coli NCTC 10418, AG100, AG102 were used as controls for MIC testing.

For Campylobacter, Isosensitest agar (Oxoid CM471) supplemented with 5% defibrinated horse blood was used. A suspension of bacteria in saline was made from fresh colonies to a density of MacFarland 0.5 standard. The cell suspension was then inoculated, using a multipoint inoculator, onto the agar containing dilutions of the antibiotic to be tested. Plates were incubated in a microaerophilic incubator for 24-48 h at 37°C. Campylobacter coli NCTC 11351, C. jejuni NCTC 11168 and C. jejuni NCTC 11366 were used as controls for MIC testing. The MIC value was recorded as the lowest concentration of the substance inhibiting growth.

Pulsed-field gel electrophoresis

Preparation and digestion of DNA with XbaI for PFGE and subsequent agarose gel separation was performed as previously described Liebana et al. (2004). S. Braenderup H9812 was used as a molecular marker for sizing of the restriction fragments.

Detection of antimicrobial resistance genes

The presence of vancomycin-resistant genes (vanA, vanB, vanC-1, vanC-2 and vanC-3) in enterococci isolates were screened using a multiplex PCR method as previously described (Dutka-Malen et al. 1995). The presence of the avilamycin-resistance gene (emtA) was screened for in resistant isolates using a method described by Aarestrup and McNicholas (2002); and mutations in L16 were identified by PCR and sequencing as previously described by Aarestrup and Jensen (2000).

Tetracycline-resistant E. coli (n = 73) were investigated for the presence of tetA and tetB resistant gene, as previously described (Randall et al. 2004).

Avilamycin extraction method and measurements by HPLC

Avilamycin levels in pig faeces were measured by HPLC using the method of Sunderland et al. (2004).
RESULTS

Effect of avilamycin on the native enterococci counts

There was no significant difference in the counts of presumptive enterococci isolated from the 14 pigs prior to treatment (Fig. 1; \( P > 0.1 \)). In the pigs exposed to avilamycin the counts decreased to below the detection limit of 20 CFU g\(^{-1}\) faeces during treatment and returned to pretreatment levels within 2 weeks after treatment had ceased (\( P > 0.1 \); Fig. 1). In the untreated pigs, the enterococci counts remained above 10\(^3\) CFU g\(^{-1}\) faeces for the duration of the study.

Emergence of avilamycin resistance in the Enterococci population

Isolates tested prior to treatment (\( n = 50 \)) had avilamycin MIC values ranging between 0.5 and 1 mg l\(^{-1}\). This remained the case in the control group for 11 weeks (\( n = 138 \)) after which the MIC range increased to 2–4 mg l\(^{-1}\) for all isolates (\( n = 84 \)). The avilamycin breakpoint was therefore set according to the control enterococci population distribution at 4 mg l\(^{-1}\). Prior to avilamycin treatment, no enterococci with MIC \( \geq 4 \) mg l\(^{-1}\) avilamycin were identified (detection limit 20 CFU g\(^{-1}\) faeces).

To test whether exposure to avilamycin \textit{in vivo} resulted in selection of resistant isolates, the MIC of enterococci isolated during and after treatment from the pigs exposed to avilamycin was measured (\( n = 400 \)). On day 33 of treatment (\( n = 7 \)) enterococci with an MIC value of 8 mg l\(^{-1}\) were isolated. On day 82 of treatment isolates (\( n = 8 \)) with MIC values ranging from 32 to 256 mg l\(^{-1}\) (geomean = 213 mg l\(^{-1}\)) were isolated, and these persisted at least 3 days post-treatment (Fig. 2). Two weeks post-treatment no enterococci with avilamycin MIC \( \geq 4 \) mg l\(^{-1}\) were detected in the pigs (detection limit: 20 CFU g\(^{-1}\) faeces).

Speciation and identification of resistance genes in avilamycin-resistant presumptive Enterococci

All of the enterococci isolates with MIC > 4 mg l\(^{-1}\) (\( n = 44 \)) were speciated: 37 were \( E. \text{faecalis} \) and seven were either \( E. \text{faecalis} \) or \( E. \text{faecium} \) (positive to both PCRs). The \textit{emtA} gene was present in all isolates with MIC \( \geq 32 \) mg l\(^{-1}\) (\( n = 44 \)). None carried the vancomycin-resistance gene. Four sensitive strains were speciated as \( E. \text{faecium} \) and none were carrying the \textit{emtA} gene.

MIC profiles of enterococci population in treated and untreated pigs

To assess whether avilamycin treatment selected for co-resistance to other agents and to assess clonality, the MIC of five unrelated antimicrobials were determined for enterococci isolated from treated (\( n = 152 \)) and control pigs (\( n = 112 \)).

In the treated pigs, 91% (\( n = 152 \)) of the avilamycin-sensitive population (MIC < 4 mg l\(^{-1}\)) had the same MIC profile to tetracycline (0.5–4 mg l\(^{-1}\)), ampicillin (0.25–0.5 mg l\(^{-1}\)), bacitracin (8 mg l\(^{-1}\)), vancomycin (1–2 mg l\(^{-1}\)) and gentamicin (8 mg l\(^{-1}\)). This was not the case with isolates from the control pigs (\( n = 112 \)); which were all sensitive to avilamycin; MIC < 4 mg l\(^{-1}\) at every sampling point there were at least five different MIC profiles (data not shown).

Resistant isolates with avilamycin MIC values of 8 mg l\(^{-1}\) (isolated from treated pigs only) all had the same MIC profile: tetracycline (32 mg l\(^{-1}\)), ampicillin (0.5–1 mg l\(^{-1}\)), bacitracin (8 mg l\(^{-1}\)), vancomycin (0.5–1 mg l\(^{-1}\)), and gentamicin (4–8 mg l\(^{-1}\)).

Highly resistant isolates with avilamycin MIC ≥ 32 mg l\(^{-1}\) (\( n = 36 \); isolated only in the treated pigs) fell into two MIC profiles which differed only in their resistance to tetracycline: 16 had a tetracycline MIC ranging between 8 and 16 mg l\(^{-1}\) and whilst the MIC of the other 17 ranged

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from 0·25 to 0·5 mg l⁻¹. Both groups had identical MIC values for ampicillin (0·25–0·5 mg l⁻¹), bacitracin (8 mg l⁻¹), vancomycin (1–2 mg l⁻¹) and gentamicin (8–16 mg l⁻¹).

**Effect of avilamycin on Salmonella and Campylobacter**

No *Salmonella* were detected in any of the pigs prior to oral inoculation with *S. Typhimurium DT104* (detection level: 0·5 CFU g⁻¹ faeces).

The effect of avilamycin on the native *Campylobacter* and inoculated *S. Typhimurium DT104* was investigated. No difference in the total counts (CFU g⁻¹ faeces) of either population could be seen when comparing control pigs and pigs treated with avilamycin.

All *S. Typhimurium* re-isolated (*n* = 350) had an MIC > 256 mg l⁻¹ for avilamycin. Their MIC profile to unrelated antimicrobials was also measured and values were identical when comparing the two groups of pigs: nalidixic acid (2 mg l⁻¹), chloramphenicol (128 mg l⁻¹), ampicillin (256 mg l⁻¹), tetracycline (32 mg l⁻¹), sulphonamide (256 mg l⁻¹), streptomycin (64 mg l⁻¹).

**Avilamycin concentration in the faeces**

Avilamycin was measured in faeces of all pigs during treatment (Fig. 4), and for 3 days post-treatment. The concentration of avilamycin was initially 0·6 mg kg⁻¹ faeces (avilamycin B) and 4 mg kg⁻¹ faeces (avilamycin A) within 24 h of treatment. Levels gradually increased and stabilized at 1 and 7·5 mg kg⁻¹, by day 61 and until the last day of treatment on day 83. A week after treatment had ceased neither metabolites were detected (detection limits, avilamycin B: 0·2 mg kg⁻¹ and avilamycin A: 0·9 mg kg⁻¹). No avilamycin A or B were detected in the faeces from the control pigs.

**DISCUSSION**

We have confirmed using a live pig model that avilamycin selects for avilamycin resistance in the native enterococci population. The resistance gene was characterized as the mutation *entA*, an rRNA methyl transferase known to

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**Enteric *E. coli* population**

Isolates from the enteric *E. coli* population (*n* = 700) were screened for changes in their MIC resistance profile to six unrelated antimicrobials and for resistance to cyclohexane. There were no changes in the total count, or in the MIC values for avilamycin (MIC ≥ 128 mg l⁻¹), nalidixic acid (2–4 mg l⁻¹), chloramphenicol (2–8 mg l⁻¹), erythromycin (32–64 mg l⁻¹), trimethoprim (0·06–0·125 mg l⁻¹), ampicillin (2–4 mg l⁻¹), and cyclohexane (all sensitive) before, during and post-treatment.

Tetracycline MICs ranged from 0·25 to 256 mg l⁻¹ in both the control and treated pigs. *E. coli* highly resistant to tetracycline (MIC ≥ 64 mg l⁻¹) were isolated from both treated and control pigs at every sampling point. In the control pigs, these represented between 8 and 20% of the *E. coli* population throughout the study. This was also the case in the treated pigs until days 47 and 62 of treatment when 44 and 76% of isolates respectively had a tetracycline MIC ≥ 128 mg l⁻¹ (Fig. 3). To define whether this high number of resistant *E. coli* was the result of a single dominant clone, API profiles were made on *E. coli* isolates with MIC ≥ 128 mg l⁻¹ (*n* = 73) from the treated pigs and five API profiles were identified. One profile (5004562) was dominant and all isolates (*n* = 25) with this profile also had the same PFGE profile, an MIC of 2 mg l⁻¹ and carried *tetB* gene. This clone continued to dominate the tetracycline-resistant *E. coli* population of the treated pigs even once the latter was no longer dominant (3 days post-treatment). All API profiles found in the treated pigs were also found in the control pigs, however the control pigs had greater number API profiles, suggesting greater population diversity.

Before treatment, the *tetA* gene was the dominant tetracycline-resistance gene. This continued to be the case in the control group. However in the treated pigs, both during and post-treatment the *tetB* gene was in 89% of tetracycline-resistant isolates.
confers high resistance to avilamycin (Mann et al. 2001). No mutations in the ribosomal protein L16, also linked with resistance to the oligosaccharides (Aarestrup and Jensen 2000) were identified in any of the resistant isolates. The cmtA mutation was located in more than one strain during and only 1-week post-treatment. However no resistant isolates were detected in the pigs from 2 weeks after treatment had ceased, indicating <0.03% resistance present from 2 weeks post-treatment onwards. This suggests that resistant isolates were unable to persist once selective pressure was removed or if they did so, they persisted at numbers below the detectable limits of the bacteriological methods used. Therefore, our data supports the hypothesis that by introducing withdrawal times the risk of resistant isolates becoming carcass contaminants, moving up the food chain and infecting man could be minimized.

Various studies have investigated the link between resistant isolates in food animals and humans. Aarestrup et al. (2000a) found similar resistance patterns and resistance genes between enterococci isolated from humans, broilers and pigs, indicating that transmission of resistant enterococci takes place between food animals and humans. However, all human and pig isolates tested were susceptible to avilamycin whilst 35% of isolates from broilers were resistant (Aarestrup et al. 2000a). In another study they investigated the epidemiology over time of occurrence of avilamycin resistance in enterococci and found very limited number of resistant isolates from pigs compared with up to 80% resistance in the poultry industry. That particular study only showed an association with the use of avilamycin and occurrence of enterococci resistance in broilers (Aarestrup et al. 2000b).

Davies and Roberts (1999) also investigated the role food animals fed avilamycin had on the increase of resistance in clinical isolates. In their study too, no avilamycin-resistant isolates were identified on treated pig carcasses when sampled at a commercial abattoir. They concluded that this could be because of the long ‘withdrawal time’ period as the avilamycin had only been fed as a starter ration to the pigs they were investigating.

In this study, the resistant enterococci were only detected 82 days (ca 3 months) into treatment and were no longer detected 2 weeks post-treatment. Compared with emergence and persistence of resistance observed in the same animal model when testing various therapeutic agents in previous studies, we argue that emergence of resistance in enterococci as a result of avilamycin was a slow process and persistence of resistance was short term (Delsol et al. 2003, 2004a,b). This observation correlates with the findings the various studies described above, which so far support the hypothesis that a regulated use of the growth promoter avilamycin could minimize the danger of resistant isolates moving up the food chain and infecting man. This solution has been perceived as safer than banning completely growth promoters by some (Casewell et al. 2003).

The effect of avilamycin on the native E. coli population and the inoculated S. Typhimurium DT104 was also monitored in an attempt to assess whether this growth promoter could induce stresses promoting the expression of efflux pumps, a mechanism of resistance shared by bacteria expressing a multiple antibiotic resistance (MAR) phenotype. Cyclohexane resistance has been linked with upregulation of efflux mechanisms conferring the MAR phenotype (White et al. 1997) and consequently was used to identify any MAR E. coli and S. Typhimurium DT104 isolates, however none were detected during or post-treatment.

Nonetheless our results did indicate that avilamycin indirectly affected the E. coli population. All E. coli tested had an avilamycin MIC > 128 mg l⁻¹ confirming these were all intrinsically resistant to this growth promoter. However, in the treated pigs, the tetB gene (which encodes for an efflux pump with particularly high affinity for tetracycline as resistant isolates with this pump all have an MIC ≥ 64 mg l⁻¹) was dominant in the tetracycline-resistant E. coli both during and post-treatment. TetB positive E. coli also dominated the E. coli population at two sampling points during treatment. API and PFGE profiles confirmed this was not the result of a single successful clone but at least 5. This was not seen in the untreated pigs, where the tetA resistance gene dominated the resistant population although all tetB E. coli strains identified in the treated pigs were also present in the untreated pigs. Whether avilamycin had an effect on the E. coli population, activating an efflux mechanism, remains a hypothesis, which merits its own full investigation and statistical validation. Incidentally, similar observations have been made by Blake et al. (2003), who found that the tetB gene dominated the resistant E. coli population isolated from intensively reared pigs, whilst the tetA and tetC genes dominated in organic pigs (Blake et al. 2003).

Unlike E. coli, no changes in the Salmonella and Campylobacter populations were detected during or post-treatment. S. Typhimurium was intrinsically resistant to avilamycin, while the Campylobacter population was formed of both isolates with avilamycin MIC ≤ 16 mg l⁻¹ and isolates with MIC ≥ 32 mg l⁻¹. No changes in the MIC profiles were detected as a result of treatment, in either population, suggesting avilamycin had no effects on these bacteria. Evans and Weneger (2003) reported a decrease in Salmonella in broilers, swine, pork and chicken meat following a ban on growth promoters in Denmark in 1998 but saw no changes in the prevalence of Campylobacter in broilers. In our case no changes in CFU counts were noted in either populations.

The excretion of avilamycin by swine has been determined as 93% in faeces (Magnussen et al. 1991). Therefore our HPLC measurements reflected accurately the levels to which the microflora was exposed. Avilamycin could still be measured from pigs 3 days post-treatment, although levels had gone down from 7·5 and 1·1 mg kg⁻¹ (last day of treatment) to 0·7 and 0·05 mg kg⁻¹ for avilamycin A and B respectively. This correlated well with the microbiological investigations, which only continued to isolate resistant enterococci 3 days post-treatment. The mechanism by which a growth promoter works is believed to involve a reduction of the intestinal bacteria, which in turn reduces the competition with the host for nutrients and reduces the number of pathogens (Jensen 1998; Corpet 2000). This correlates with our study as in the treated pigs the number of pathogens decreased during treatment and the E. coli population decreased during treatment and the E. coli population was composed of a smaller number of clones than the E. coli population in the control pigs.

In conclusion, avilamycin does select for resistance in the enteric enterococci in the pig. However these only persisted a week post treatment, and by simply introducing a withdrawal time, the risk posed by these resistant isolates to human health could be greatly minimized.

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