Potential effects of carryover of antimicrobials in pig feed

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Introduction

Following the recent Dutch comments on the possibility of banning the use of medicated feed premixes to reduce antimicrobial use and then the assertion that the carryover of antimicrobials from one feed to the following feed might also cause the induction of antimicrobial resistance in commensal and potentially zoonotic bacteria, it was considered interesting to look at the potential risks that might be involved. Pig premix inclusion levels for common antimicrobials were used and where data was not available a model to assess colonic contents concentration and that achieved in the small intestine (Burch, 2007) was used to compare the antimicrobial concentrations with their minimum inhibitory concentrations (MICs) against commensals, Escherichia coli or Enterococci and zoonotic Campylobacter coli primarily from pigs, to try to examine the potential risk.

Intestinal concentrations calculations

The colonic contents concentration was either available from references or were derived using a basic model. The in-feed concentration was used, less the bioavailability (absorbed amount) of the product, times the concentration ratio in the colon of approximately 1: 0.6 feed to faeces concentration. The small intestinal contents concentration (SICC) was estimated at 25% of the colon contents or faecal concentration (CCC).

Antimicrobial susceptibility patterns

For antibiotics, which were active against porcine E. coli and C. coli, examples of commensal and potentially zoonotic bacteria respectively, the susceptibility patterns were taken from Maran 2008 (2010) the Dutch report on antimicrobial susceptibility in their country.

For antibiotics, which were not active against E. coli, like the macrolides, the Enterococci results were used (E. faecalis and E. faecium) as well as C. coli. Unfortunately, the individual porcine results were not reported for the two Enterococci species but only all animal species, including isolates from pigs, broilers and cattle.

Antimicrobial comparisons

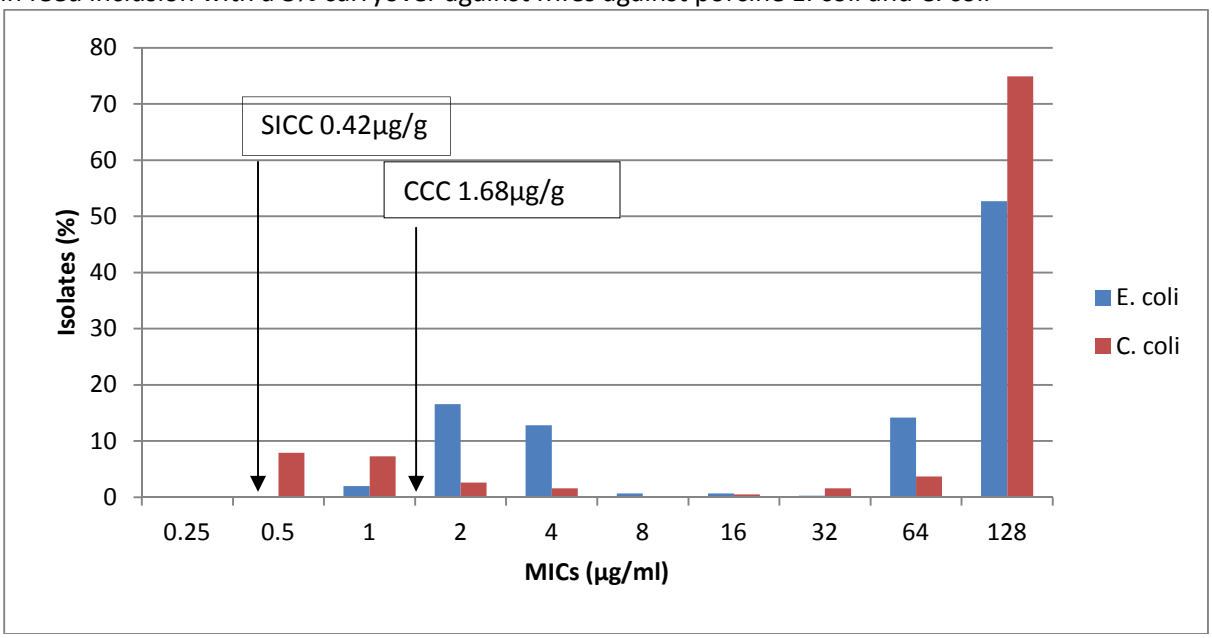
Tetracyclines (chlortetracycline)

The faecal concentration of chlortetracycline, after administration of 800ppm chlortetracycline in the feed was 112µg/g (Hansen et al, 2002). In the UK 400ppm is the more common inclusion level and therefore 56µg/g was used in the calculations. The tetracyclines are the most commonly used antibiotics in pig feed in the EU.

Table 1. Chlortetracycline colon and small intestine contents concentration at 400ppm in feed inclusion

Chlortetracycline 400ppm in feed	Inclusion level (%)	Colon contents concentration (µg/g)	Small intestine contents concentration (µg/g)
	100	56	14
	10	5.6	1.4
	3	1.68	0.42

Figure 1. Chlortetracycline colon (CCC) and small intestine contents concentration (SICC) at 400ppm in feed inclusion with a 3% carryover against MICs against porcine E. coli and C. coli



The colon contents concentration at 3% might have an impact on a very small number of isolates of both E. coli and C. coli but the small intestinal contents concentration should have no effect. Chlortetracycline can act in an anaerobic environment. There is already a substantial amount of resistance observed to chlortetracycline by both organisms above the full (100%) clinical breakpoint of 56µg/ml.

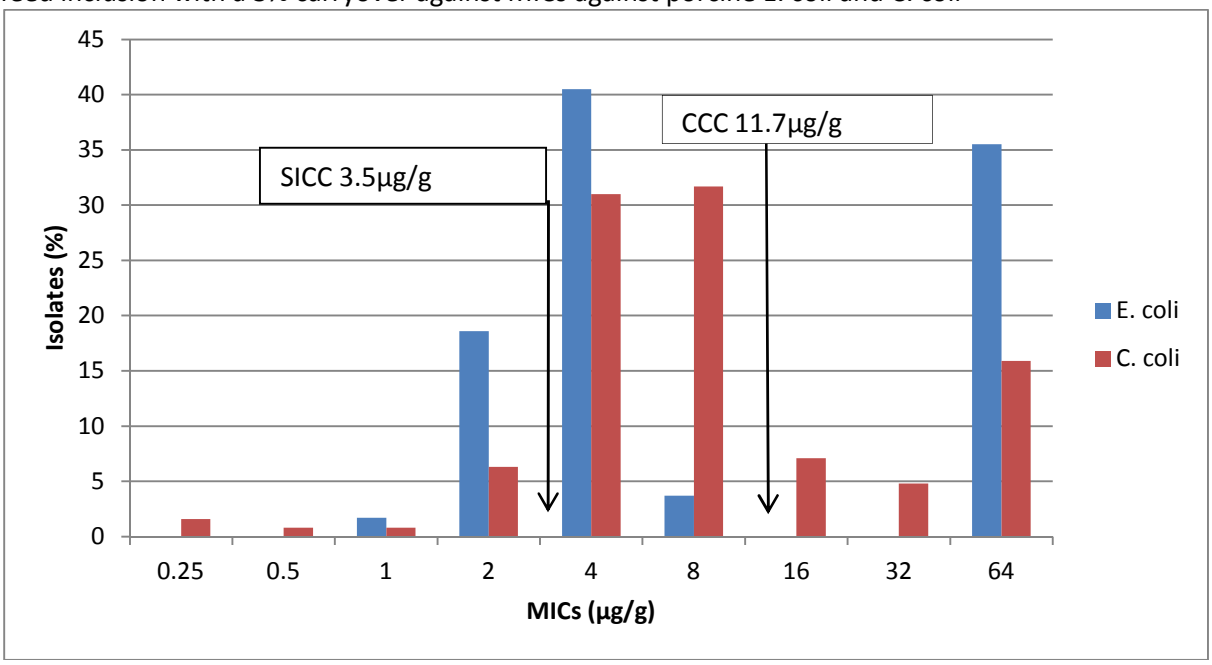
Beta-Lactams (Ampicillin/amoxycillin)

Amoxycillin is commonly administered in feed at 400ppm. It is absorbed from the gut (approximately 30%) and potentially substantial quantities can pass down into the colon contents. No published data were available so a 'worst case' scenario of 70% was used in the calculations.

Table 2. Amoxycillin colon and small intestine contents concentration at 400ppm in feed inclusion

Amoxycillin 400ppm in feed	Inclusion level (%)	Colon contents concentration (µg/g)	Small intestine contents concentration (µg/g)
	100	467	117
	10	47	11.7
	3	14.1	3.5

Figure 2. Amoxycillin colon (CCC) and small intestine contents concentration (SICC) at 400ppm in feed inclusion with a 3% carryover against MICs against porcine E. coli and C. coli



Amoxycillin concentrations in the small intestine should have a minor effect but in the colon could exert an effect on a large number of susceptible isolates of both E. coli and C. coli, providing there is no breakdown or binding of the drug on its passage down the intestine. Amoxycillin is active anaerobically, so the colon contents concentration is potentially significant.

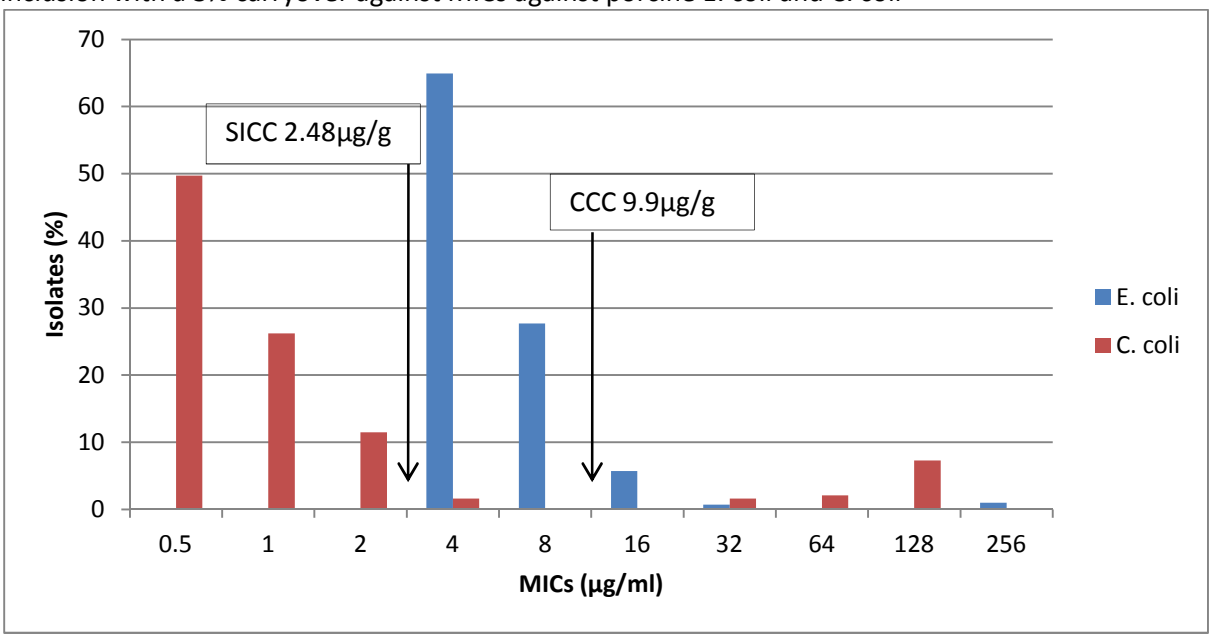
Aminoglycosides (Neomycin/Kanamycin)

Neomycin was commonly used at 220ppm. It is very similar in its mode of action to Kanamycin. It is poorly absorbed from the gut (<10%) so a figure of 90% is used in the model. It does not take into account any breakdown or binding in the gut. It is not active in an anaerobic environment so small intestine contents concentrations are probably more representative.

Table 3. Neomycin colon and small intestine contents concentration at 220ppm in feed inclusion

Neomycin 220ppm in feed	Inclusion level (%)	Colon contents concentration (µg/g)	Small intestine contents concentration (µg/g)
	100	330	82.5
	10	33	8.25
	3	9.9	2.48

Figure 3. Neomycin colon (CCC) and small intestine contents concentration (SICC) at 400ppm in feed inclusion with a 3% carryover against MICs against porcine E. coli and C. coli



The small intestinal contents concentration would have no expected effect against E. coli, but could potentially have quite a marked effect on C. coli. However, the incidence of clinical resistance is relatively small in comparison with other antimicrobials routinely used in pig medicine.

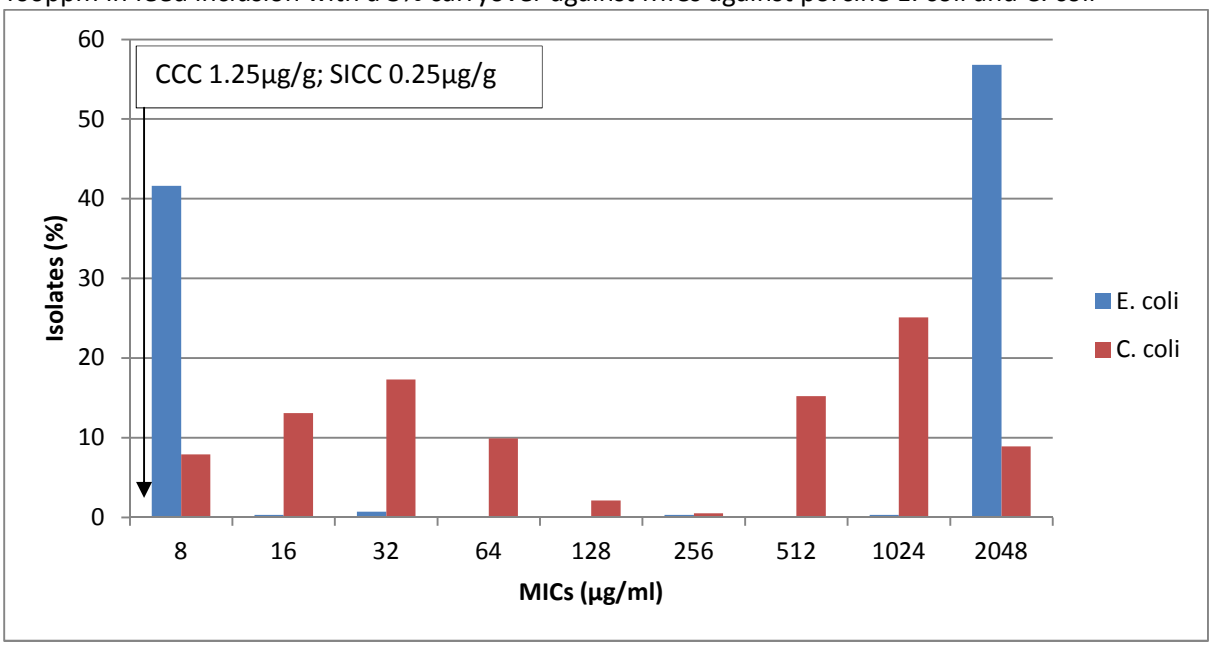
Sulphonamides (Sulphamethoxazole)

Sulphonamides are rarely used on their own in pigs but usually in combination with trimethoprim. Unfortunately, there is no combined MIC data available. Sulphamethoxazole is commonly used at 250ppm and is well absorbed (90%) so only approximately 10% will pass down the intestines into the colon.

Table 4. Sulphamethoxazole colon and small intestine contents concentration at 250ppm in feed inclusion

Sulphamethoxazole 250ppm in feed	Inclusion level (%)	Colon contents concentration (µg/g)	Small intestine contents concentration (µg/g)
	100	42	10.4
	10	4.2	0.83
	3	1.25	0.25

Figure 4. Sulphamethoxazole colon (CCC) and small intestine contents concentration (SICC) at 400ppm in feed inclusion with a 3% carryover against MICs against porcine E. coli and C. coli



The colon contents and small intestine contents concentrations are well below the MICs for both E. coli and C. coli. It is therefore considered that they will have no effect on selection for antimicrobial resistance at a 3% carryover.

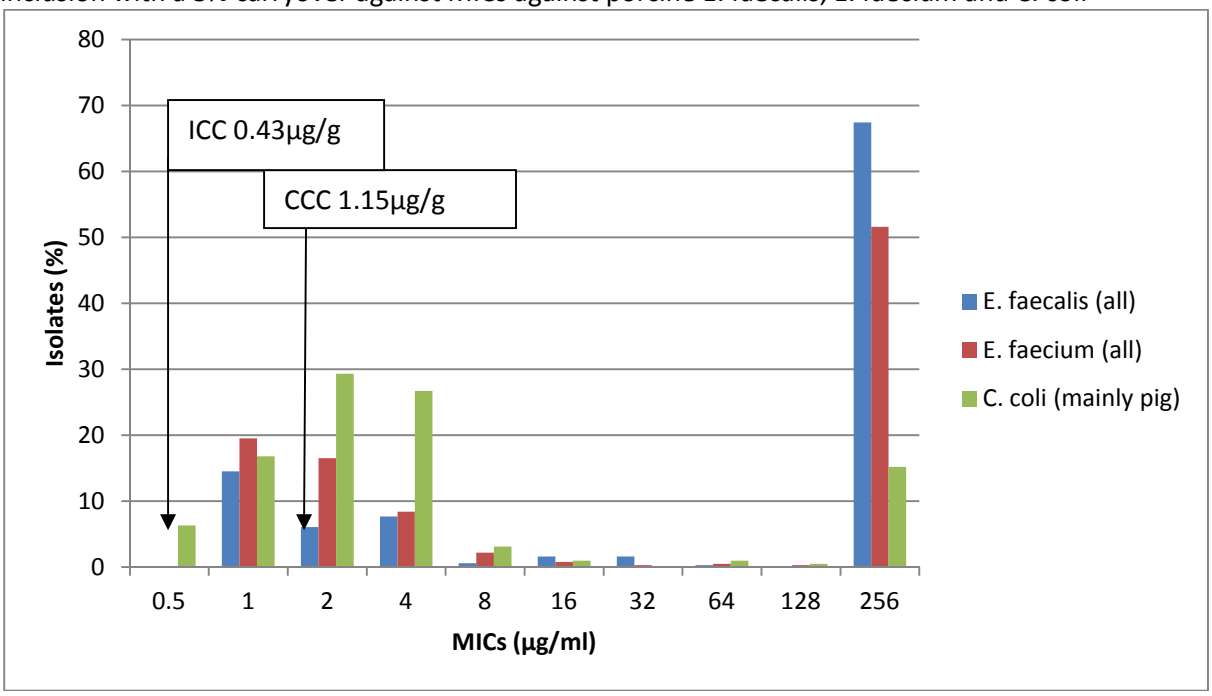
Macrolides (erythromycin; tylosin)

Erythromycin is used in the report as the representative of the Macrolide family, which includes the widely used antibiotic Tylosin. Recent gut concentration data are available (Karanikolova and others, 2010) adjusting the dose for the right inclusion rate of 100ppm concentrations in the colon contents were 38.2µg/g and in the ileum (the terminal part of the small intestine) 14.2µg/g.

Table 5. Tylosin colon and ileum contents concentration at 100ppm in feed inclusion

Tylosin 100ppm in feed	Inclusion level (%)	Colon contents concentration (µg/g)	Ileum contents concentration (µg/g)
	100	38.2	14.2
	10	3.8	1.4
	3	1.15	0.43

Figure 5. Tylosin colon (CCC) and small intestine contents concentration (ICC) at 100ppm in feed inclusion with a 3% carryover against MICs against porcine E. faecalis, E. faecium and C. coli



The ileal contents concentration at 3% carryover is below the recorded MICs. As Tylosin is anaerobically active the colon contents concentration is probably the most representative but this would appear to have only a minor effect on a few isolates of Enterococci and C. coli. This also assumes that all of the drug concentration is bioavailable and not bound to the contents at all.

Conclusions

Table 6. Summary chart of the effects of 3% carryover of antimicrobials in feed on commensal and potentially zoonotic gut flora of the pig

Antimicrobial	Small intestinal contents conc		Colon contents concentration		Comments
	E. coli	C. coli	E. coli/ Enterococci	C. coli	
Tetracyclines	0	0	+	+	Low risk
Amoxycillin	+	+	+++	+++	Modelled data only – *needs more work*
Neomycin	0	+++	0	0	Not active in anaerobic environment
Sulphamethoxazole	0	0	0	0	No risk
Tylosin	0	0	+	+	Low risk

Key: 0 = no risk; + = low risk; ++ = moderate risk; +++ = high risk

It is considered that soluble sulphonamides that are regularly used with Trimethoprim are unlikely to have an impact on both E. coli and C. coli. Neomycin could have a possible effect on C. coli isolates but resistance is not a major issue, suggesting the mode of action of the drug only induces resistance at a low rate. In many countries of the EU neomycin in feed is now not available. In comparison Chlortetracycline resistance is widespread for both E. coli and C. coli and carryover of 3% might have

a very minor impact on resistance selection in comparison with therapeutic use. Amoxycillin does have the potential to select for both E. coli and C. coli resistance, based on model data. Other beta-lactam antibiotics, like Penicillin G, are very unstable in the gut and disappear in the colon, so further work is required to develop more definitive data for amoxycillin. Tylosin would appear to be of low risk at 3% of the recommended inclusion rate in feed.

It can be concluded that generally a 3% carryover, similar to the tolerance carryover limit for anticoccidials approved by EFSA, is unlikely to result in major resistance development in comparison with regular therapeutic use. However, there is a possibility that some antibiotics, such as amoxycillin, even at low concentrations might have an impact. It is recommended that further work to determine more accurately concentrations achieved in the gut is carried out before final conclusions can be made.