Concentrations of selected antimicrobials in caecum, colon and manure of pigs due to a 3 % cross-contamination of the feed and their effect on resistance in Escherichia coli

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Introduction

Pig feed may contain up to 3% carry-over of the recommended therapeutic concentrations of antimicrobials as a result of contamination between medicated and non-medicated feed. The gut concentrations of three commercially available formulations of antimicrobials, frequently used in pig rearing, were determined through an in vivo experiment in order to investigate their possible selective effect for resistant bacteria. The effect of 1 µg/ml and 4µg/ml doxycycline on resistance selection and transfer in E. coli was investigated in vitro.

Materials and methods

1. Feed: 3 batches with a 3% carry-over level of the recommended therapeutic dose of doxycycline (DOX), sulfadiazine-trimethoprim (SULFA-TRIM) and chlorotetracycline (CTC), respectively.

2. Animal experiment

Twenty-four pigs were similarly divided into one control group and three experimental groups receiving feed containing 10 mg/kg doxycycline (1 µg/ml) in vitro for 10 days.

3. Chemical analysis

Antimicrobial concentrations were determined using in-house developed and validated LC-MS/MS methods.

4. Based on the animal experiment results (see below), we decided to investigate the selective effect of 4 µg/ml DOX (corresponding to 3% cross-contamination of the feed) and 1 µg/ml DOX (1% cross-contamination) in vitro in selected E. coli isolates.

Results

1. Mean concentrations (+/- SD) of each antimicrobial rose to a steady state on day 4 of:
   - 4 mg/kg wet weight (w.w.) (DOX)
   - 10 mg/kg w.w. for (CTC)
   - 500-700 µg/kg w.w. for (SULFA)
   - Trimethoprim: all values were below the limit of detection, so quantification was not feasible.

2. Mean antimicrobial concentrations in manure, caecum and colon content:
   - Tetracyclines: concentrations are relatively high in general and highest concentrations are found in manure.
   - Sulfadiazine: concentrations are relatively low in general and highest concentrations are found in middle colon.
   - Trimethoprim: all values were below the limit of detection, so quantification was not feasible.

3. Supplements of the medium, both with 1 µg/ml as well as with 4 µg/ml doxycycline, resulted in a strong selection of the resistant donor strain compared to the blank medium.

4. Counting of transconjugants in the supplemented media was not possible in most cases, due to the low number of transconjugants compared to the donor strain. Consequently, plasmid transfer rates could not be determined. However, in contrast with what could be expected, analysis of these results indicates that plasmid transfer rates for the selected strains are in most cases lower in the supplemented media compared to the blank medium.

5. The results show that plasmid transfer rates (i.e. with rifampicin resistant recipient strain)

6. Conclusions

The results of the animal experiment show that the poor oral bioavailabilities of tetracyclines may result in rather high concentrations in caecum, colon and manure, even at 3% cross-contamination of the feed. As expected, the high oral bioavailabilities of sulfadiazine and trimethoprim appear to result in very low gut concentrations. The in vitro research on the effect of 1 µg/ml and 4µg/ml doxycycline showed that both concentrations have a clear selective effect on the resistant donor strain. This effect seems higher at 1 µg/ml compared to 4 µg/ml, which could possibly be due to the fact that 4 µg/ml is the ECOF value (Euca) for doxycycline in E. coli. In contrast to what one would expect, plasmid transfer rates appeared lower in supplemented media compared to the blank medium. Further research is needed to quantify these transfer rates.

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