



Comparison of AMR-development in rectal *Enterobacteriaceae* from piglets during phenoxymethylpenicillin (Phenoxyphen[®]) or amoxicillin drinking water treatment

Introduction

Antibiotic treatment and metaphylaxis can be essential for animal welfare during life threatening and rapidly spreading infections like *Streptococcus suis* in swine. However, a negative aspect of such intervention is the selection for less sensitive bacteria and therefore risk of induction of antimicrobial resistance (AMR) in target pathogens as well as in the microbiome of treated animals. It is assumed that oral treatment with narrow-spectrum antibiotics will induce less AMR in *Enterobacteriaceae* populations from the gut microbiome compared to broad-spectrum antibiotics. The aim of this study is to test this hypothesis.

Materials & Methods

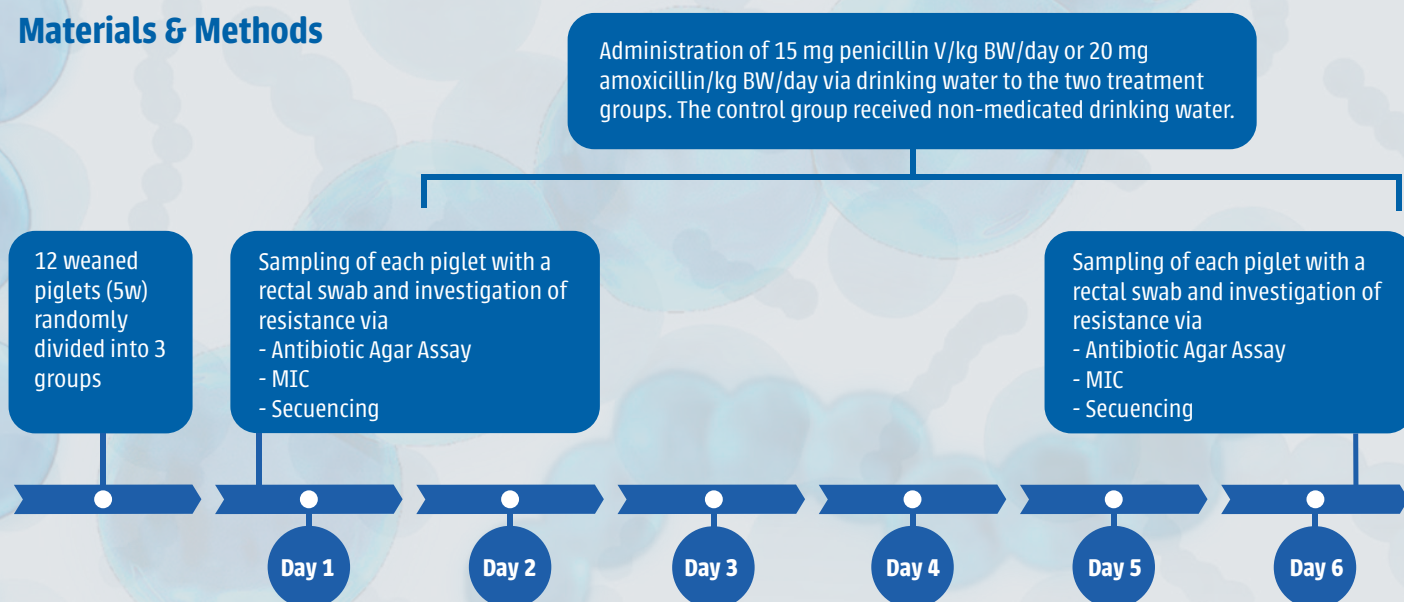


Figure 1: Overview of the study design.

The study was conducted in a controlled, randomized and blinded design. Isolated *Enterobacteriaceae* populations were phenotypically and genotypically examined for AMR against amoxicillin, trimethoprim/sulfonamide combination and doxycycline. Resistance was determined by several laboratory methods including an antimicrobial agar assay, a minimum inhibitory concentration test (16 colonies per sample) and by long-read nanopore shotgun sequencing (20 colonies per sample).

Results

The water consumption of the piglets was on average higher than expected from the pre-trial measurements. This resulted in an average daily dose of 18.1 mg phenoxymethylpenicillin/kg BW and 23.2 mg amoxicillin/kg BW. *Escherichia coli* was the most abundant bacterial species identified from all *Enterobacteriaceae* (mean 97.2% (\pm 3.1%)).

Antibiotic agar assay: *Enterobacteriaceae* from piglets treated with amoxicillin showed a statistically significant increase in resistance against amoxicillin during treatment compared to pre-treatment ($p = 0.047$).

Long-read nanopore shotgun sequencing: *Enterobacteriaceae* from piglets treated with amoxicillin showed a statistically significant increase in beta-lactam resistance genes (TEM) during treatment compared to pre-treatment ($p = 0.027$).

	Antibiotic Agar Assay		Beta-lactam (TEM) genes	
	Day 1	Day 6	Day 1	Day 6
Control	3.6%	0.2%	0.5%	0.02%
Penicillin V	5.7%	51.6%	0.2%	1.16%
Amoxicillin	10.1%	84.4% (p=0.047)	0.1	1.9% (p=0.027)

Table 1: Resistance of *Enterobacteriaceae* (mainly *Escherichia coli*) against amoxicillin before treatment at day 1 and at the last day of treatment (day 6) (Control = negative control group; Penicillin V = phenoxymethylpenicillin treatment group; Amoxicillin = amoxicillin treatment group). Antibiotic Agar Assay: percentage of *Enterobacteriaceae* colonies growing on amoxicillin supplemented medium compared to total colonies on plates without amoxicillin. TEM-genes: relative abundance of beta-lactam (TEM) genes. Bold percentages show a statistically significant increase in percentages between day 1 and day 6 ($p < 0.05$).

In contrast, the control group and the phenoxymethylpenicillin treated pigs showed no statistically significant difference in AMR in any of the performed tests before and during treatment. Figure 2 shows the results of the MIC-analyses before and during treatment. Again, the *Enterobacteriaceae* from the amoxicillin treated group showed the highest increase in resistance. Our results showed no increase of resistance against doxycycline and trimethoprim/sulfonamide combination (data not shown).

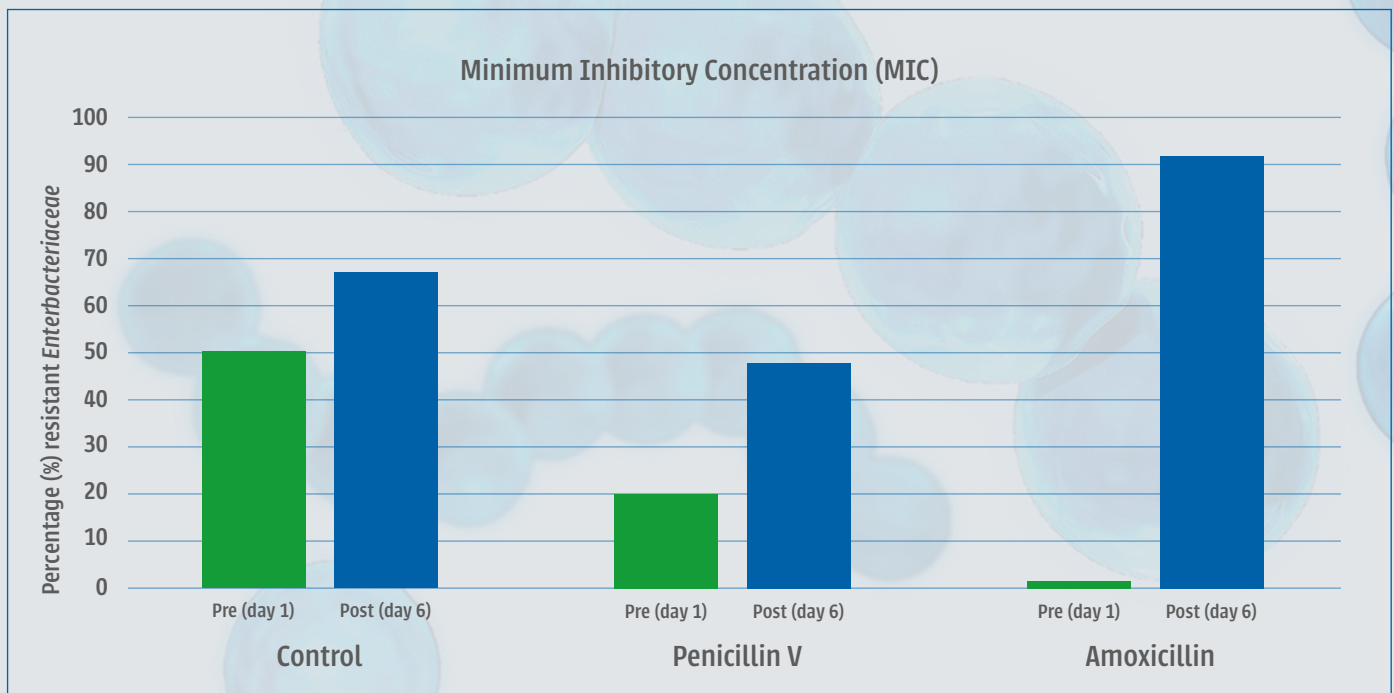


Figure 2: Results of the MIC-sensitivity tests. The percentage of resistance shows the percentage of *Enterobacteriaceae* with a MIC > the epidemiological cut-off value of 2 µg/ml. Green bars represent the percentage before the start of the treatment (day 1). Blue bars represent the percentage at the last day of treatment (day 6).

Conclusion

Streptococcosis is a multifactorial disease. The preferred control of the disease is via (biosecurity) management and vaccination, but these interventions do not always prevent clinical symptoms. During outbreaks, antibacterial treatment and metaphylaxis need to be started quickly, and drinking water medication is a suitable option. This study confirms our hypothesis that treatment with the narrow-spectrum penicillin V, induces less resistance in gram-negative *Enterobacteriaceae* from the intestinal microbiome, compared to broad-spectrum amoxicillin treatment. The new innovative registration of Phenoxyphen® is therefore a valuable addition to the treatment options for *S. suis* and supports Good Veterinary Practice and the One Health-concept in pig farming.

