

## **IMPACT OF PEN CLEANING AND THE PROPHYLACTIC USE OF ANTIMICROBIAL AGENTS ON THE PORCINE FAECAL RESISTOME.**

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### **INTRODUCTION**

Post-weaning diarrhoea is a major concern on commercial pig farms, as it accounts for losses of up to 17 % of newborn piglets within the European Union. Infection with causative agents usually occurs at weaning, during which the piglets endure different stressors. Although the prophylactic use of antimicrobials such as zinc oxide and antibiotics can help to prevent the infection of piglets, new European Union legislation will prohibit the prophylactic use of antimicrobials from 2022, to reduce the development of antimicrobial resistance (AMR). However, it is still not fully understood how antimicrobial use (AMU) can affect the porcine resistome. Moreover, further insights into the effect of AMU on the porcine resistome may facilitate the development of alternative disease prevention methods on commercial pig farms. This study aimed to elucidate relations between AMU and faecal AMR in a controlled environment. Furthermore, different cleaning methods were assessed to determine their impact on the porcine resistome.

### **METHODOLOGY**

Three independent trials were conducted in a controlled environment, each with three different treatment groups [apramycin, zinc oxide, control (no antimicrobials)]. In each trial, piglets were weaned at the beginning of the trial (at four weeks of age) and fed for approximately one week with antimicrobial-containing or control feed, according to their treatment group. Additionally, in each trial, different cleaning approaches (not washed; pre-soaked + power washed; pre-soaked + power washed + disinfected; not pre-soaked + power washed + disinfected; or pre-soaked + power washed + detergent applied + disinfected + dried) were used for the trial pens before the piglets entered. To determine the impact of pen cleaning and antimicrobial feed on the porcine resistome, fresh faecal samples were taken approximately one week after treatment and analysed using shotgun metagenomics. In various pre-processing steps, raw sequencing data were cleaned and host sequences removed before cleaned sequences were aligned against the ResFinder database to determine the porcine resistome. Alpha diversity and beta diversity were analysed using RStudio and the vegan package.

### **RESULTS**

Alpha diversity measures of antimicrobial resistance genes (ARGs) showed no statistically significant differences between the treatment groups or cleaning methods. AMR was commonly observed to tetracycline, aminoglycoside, beta-lactam and MLSP (macrolide, lincosamide, streptogramin, pleuromutilin) antibiotics in all treatment and cleaning groups. Distinct clusters of treatment groups and cleaning methods were observed for beta diversity measures, which were evaluated using non-metric dimensional scaling (NMDS). Thus, the use of different treatments explained 12.9 % of the variation in the NMDS analysis, while different cleaning methods accounted for 14.7 % of the variation.

## DISCUSSION

Alpha diversity measures of the three independent trials with different treatment and cleaning methods showed no significant differences between them. However, a clear impact of treatment and cleaning on beta diversity was observed. These differences were seen in faecal samples from piglets, which were collected seven days post-treatment with antimicrobial-containing feed, which highlights the rapid emergence of AMR in association with AMU. Furthermore, cleaning also contributed to the AMR profiles in this study, as was observed for the piglets fed with the control feed in combination with intensive cleaning. Consequently, the use of disinfectants and detergents can also be selective pressures for AMR. It is important to note that this study was conducted within a controlled environment, meaning that there is no background of AMU as there may be on a commercial pig farm. Nevertheless, this study suggests that different on-farm cleaning methods can also impact the structure of the porcine resistome. Therefore, farm management practices and targeted risk reduction measures could offer possibilities to influence the on-farm resistome. However, further research in commercial settings will be required.