Summary: Evaluation of the Impact of Antimicrobial Use Protocols in Porcine Reproductive and Respiratory Syndrome Virus-Infected Swine on Phenotypic Antimicrobial Resistance Patterns

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Key Points:

- Antimicrobial protocols of differing intensities of exposure were compared with a control group with minimal antimicrobial exposure and animals were followed for 149 days.
- Fecal samples revealed most *E. coli* isolates were resistant to between one and 11 antimicrobials with 19 different observed resistance profiles, while most *Enterococcus* isolates were resistant to between two and nine antimicrobials and 27 resistance patterns were observed.
- Few significant differences between groups or over time were observed.

Antimicrobial resistance (AMR) may impact both animal health, through loss of antimicrobial efficacy to treat animal pathogens, and human health via transmission of resistant organisms from animals to people. The contribution of antimicrobial use (AMU) in animals to the burden of AMR in human medicine remains a subject of longstanding and unresolved debate. Regardless of the ongoing uncertainties surrounding the potential impact upon public health, veterinarians should seek to refine and optimize their prescribing practices. In food animals, one aspect that has been largely ignored is the extent to which the timing of antimicrobial exposure of food animals in relation to harvest may influence the profile of resistant pathogens entering the food supply chain. In commercial swine production, the need for antimicrobials is typically greatest in weaned pig populations, which means that much exposure to antimicrobials occurs several months prior to marketing. Thus, longitudinal studies that involves repeated observations over a period of time to assess the effects of AMU are needed.

Given the importance of porcine reproductive and respiratory syndrome virus (PRRSV) for driving both swine health and AMU, there is a need to better understand how AMR may be affected by AMU protocols used in PRRS outbreaks. This longitudinal study was undertaken to compare animal health and AMR outcomes of antimicrobial treatment protocols in PRRSV challenged pigs. The study compared phenotypic resistance prevalence and patterns in Gram-negative (*E. coli*) and Gram-positive (*Enterococcus* spp.) commensals. The primary objective of this study was to evaluate how the AMR prevalence in fecal *E. coli* and *Enterococcus* spp. would be affected by exposure to antimicrobials, and to describe temporal patterns of AMR prevalence from weaning until market.

Based on practical approaches used to treat moderate to severe PRRSV-associated secondary bacterial infections, two antimicrobial protocols of differing intensities of exposure [44.1 and 181.5 animal-treatment days per 1000 animal days at risk (ATD)] were compared with a control group with minimal antimicrobial exposure (2.1 ATD). Litter matched pigs (n = 108) with no prior antimicrobial exposure were assigned randomly to the treatment groups. Pen fecal samples were collected nine times during the wean-to-finish period and cultured for *Enterococcus* spp. Antimicrobial-susceptibility testing was conducted using NARMS Gram-negative and Gram-positive antibiotic panels.

Of the 324 composite fecal samples cultured, *E. coli* was isolated from 307 (94.7%) and *Enterococcus* spp. from 292 (90.1%) samples. At the first sampling after weaning (i.e., before any exposure to antimicrobials), 30 of the 33 *E. coli* isolates were resistant to at least one antimicrobial, with individual isolates resistant to up to 11 antimicrobials. None of the 36 *Enterococcus* isolates at that sampling were pansusceptible, with individual isolates resistant to between two and nine antimicrobials. Among all fecal *E. coli* isolates, resistance was most common to tetracycline, ampicillin, and streptomycin. The sole significant finding for *E. coli* isolates was with streptomycin between treatment groups. The odds of resistance to streptomycin were significantly higher ($P < 0.0001$) in the Moderate group than in the Minimal and the Intensive groups. Among the *Enterococcus* isolates, resistance was most common to erythromycin, lincomycin, quinupristin-dalfopristin, tetracycline, and tylosin. No significant differences were identified between treatment groups.

Among the 307 *E. coli* isolates, 30 isolates (10.1%) were pansusceptible to the Gram-negative panel of antimicrobials. Of the 182 *E. coli* isolates resistant to more than one antimicrobial, 19 different resistance profiles were observed. These resistance profiles most frequently included resistance to tetracyclines, penicillins, and aminoglycosides. Among the 292 *Enterococcus* sample, only 9 (3.1%) were pansusceptible to the Gram-positive panel of antimicrobials, and another 33 (11.3%) were resistant to a single antimicrobial. Among the 250 Enterococci resistant to more than one antimicrobial, 27 resistance patterns were observed. These resistance patterns most frequently included resistance to tetracyclines, lincosamides, macrolides, and streptogramins.

Despite a 65.3-fold difference in ATD between groups of pigs, with the exposures concentrated in a 25 day window within the 149 day study period, we found few significant differences between groups, or over time, in the likelihood of phenotypic AMR in commensal bacteria cultured from feces. Furthermore, these significant differences observed could not be easily reconciled with the specific antimicrobials administered or the timing of the exposure of pigs. The sole difference between groups was for streptomycin in *E. coli*, yet no aminoglycoside antimicrobials were administered in the study, and resistance was higher in the Moderate group than the Minimal and Intensive groups. In all instances the odds of AMR differed between time points, the higher AMR prevalence occurred at the first sampling prior to any antimicrobial exposure. These unanticipated outcomes bear witness to the complexity of use-resistance relationships. Overall, resistance patterns were remarkably stable between the treatment groups over time, and the differences observe could not be readily reconciled with the antimicrobial exposures, indicating the likely importance of other determinants of AMR at the population level.

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