**Objective:**To determine if different disease-relevant antimicrobial treatment protocols are associated with differences in the fecal resistome of growing pigs.

**Study Design:**

**Animals:**108 piglets divided into three groups (36 per group):

1. **Group 1:** Control group: No PRRS virus, minimal antibiotics
2. **Group 2:** PRRSv-challenge group with moderate antibiotics
3. **Group 3:** PRRSv-challenge group with intensive antibiotics

**Sample Collection:**Individual fecal samples collected at six time points (from weaning to market), combined by pen within each time point and treatment group (N=216 samples total).

**Analysis:**Genomic DNA extractions followed by shotgun metagenomic sequencing.

**Tasks:**

1. Load output files and create a phyloseq object for analysis.
2. Remove “RequiresSNPConfirmation” prior to analysis.
3. Summarize basic QC metrics:
   * Total raw reads, mean [range], median
   * Mean quality score [range]
   * Number of raw reads aligned to the resistome by treatment group, negative control, and positive control samples

Run statistical models to check for differences in average raw reads between treatment groups.

1. Create a stacked bar chart showing the proportion of host and non-host reads.
2. Calculate the total number of unique AMR gene groups (ARGs), mechanisms, classes, and types found across all samples.
3. Calculate alpha diversity (richness, Shannon diversity) at the class, and group levels and create box plots by treatment groups.
   * Run statistical models to test if these values differ between treatment groups.
4. Assess if the overall resistome (at class and ARG group levels) differs between treatment groups:
   * Are treatment group samples and negative/positive samples clustered together (i.e., is the overall resistome similar)?
5. Perform statistical tests to determine if overall resistome composition at the class and ARG group levels differs by treatment group.
   * Determine how much variation is attributed to the treatment group. What about variation is attributed to the sampling points?
6. Plot the relative abundance of the resistome at the class level by treatment group and identify the most abundant class across treatment groups.
7. Determine which ARG groups were differentially abundant between treatment groups (i.e., significant increases or decreases in the abundance of ARG groups between treatment groups) [optional]
8. Summarize key findings.