

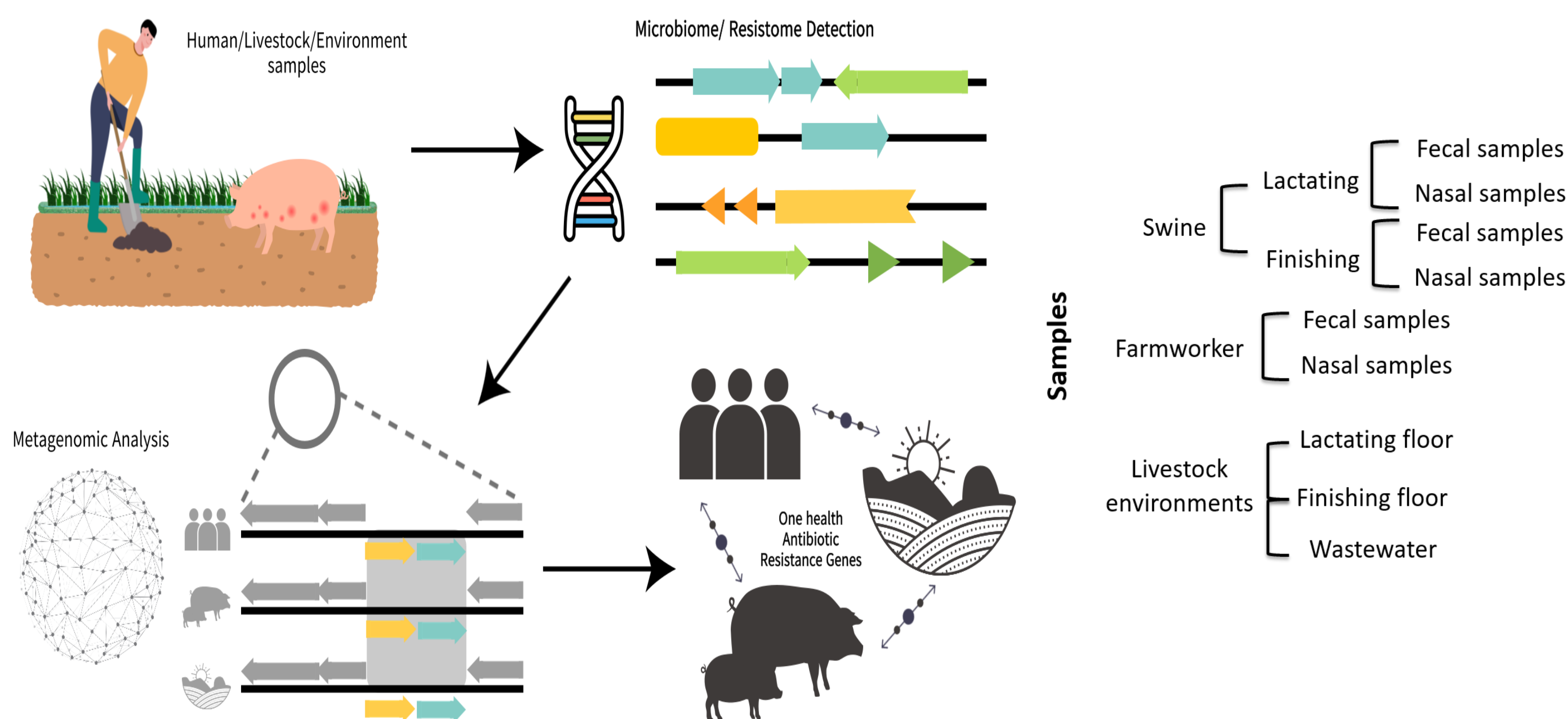
Human-Swine-Environment Microbiome/Resistome Analysis

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Abstract

Interconnected human-animal-environment can contribute to the emergence and propagation of antibiotic resistance, which can pose a great threat to human health¹⁾. Therefore, an effective response to antibiotic resistance requires a one-health approach that recognizes people, animals, and the environment collaboratively²⁾. We analyze the distribution and correlation of the resistome of livestock farm samples using shotgun metagenomic sequencing. Bacteroidota is the most common bacterial phylum detected in the sample. The bacterial community was significantly different according to environment, organism, environment site, and site. The most common ARG(Antibiotic resistance gene) class in all samples is aminoglycoside resistance. Except for tetracycline, nasal samples have more ARG than fecal samples. There is a significant difference in ARG in the human-swine-environment, but there is no significant difference in human-swine, human-environment. The ARG of the sample has a positive correlation with each other. Therefore, antibiotic resistance genes can be shared and propagated.

Methods & Materials



- Samples are collected in swine, farmworker, and farm environment and sequenced with illumina NovaSeq.
- Metagenomic analysis was conducted in the order of quality control, host removal, assembly, ORF(Open reading frame) prediction, and reconstruction MAG(Metagenome-assembled genome). A CARD database was used for resistome analysis.
- Based on the metagenome, we identify bacteria and antibiotic resistance genes that exist in the human-swine-environment and analyze their patterns and correlations.

Bacterial distribution and dissimilarity

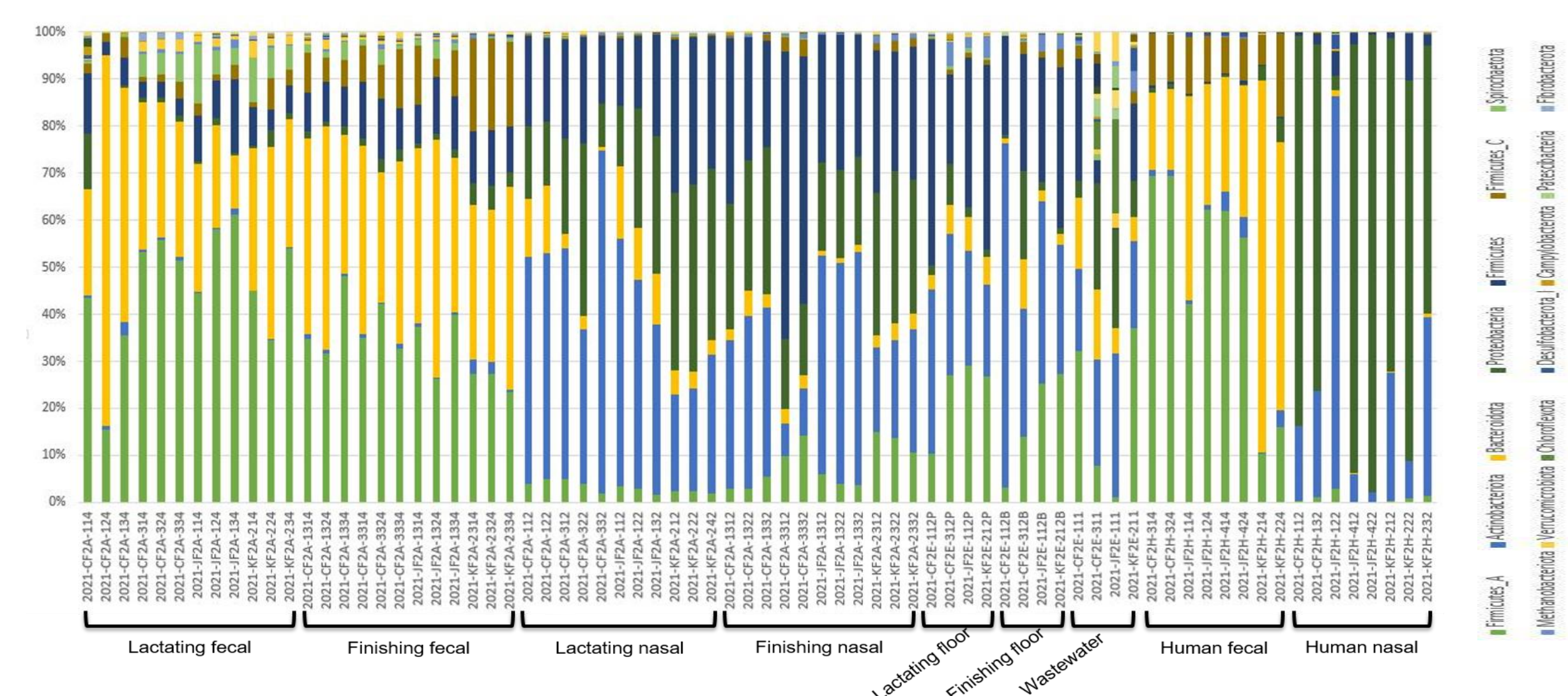


Fig 1. Distribution of bacterial phylum

- The relative abundance of bacterial phylum in the total samples according to the environment.
- The most dominant phylum in the total sample is Bacteroidota. In the fecal sample, the most dominant phylum is Firmicutes_A. Actinobacteriota is the most dominant in the swine nasal and environment. Proteobacteria is the most dominant in the human nasal.

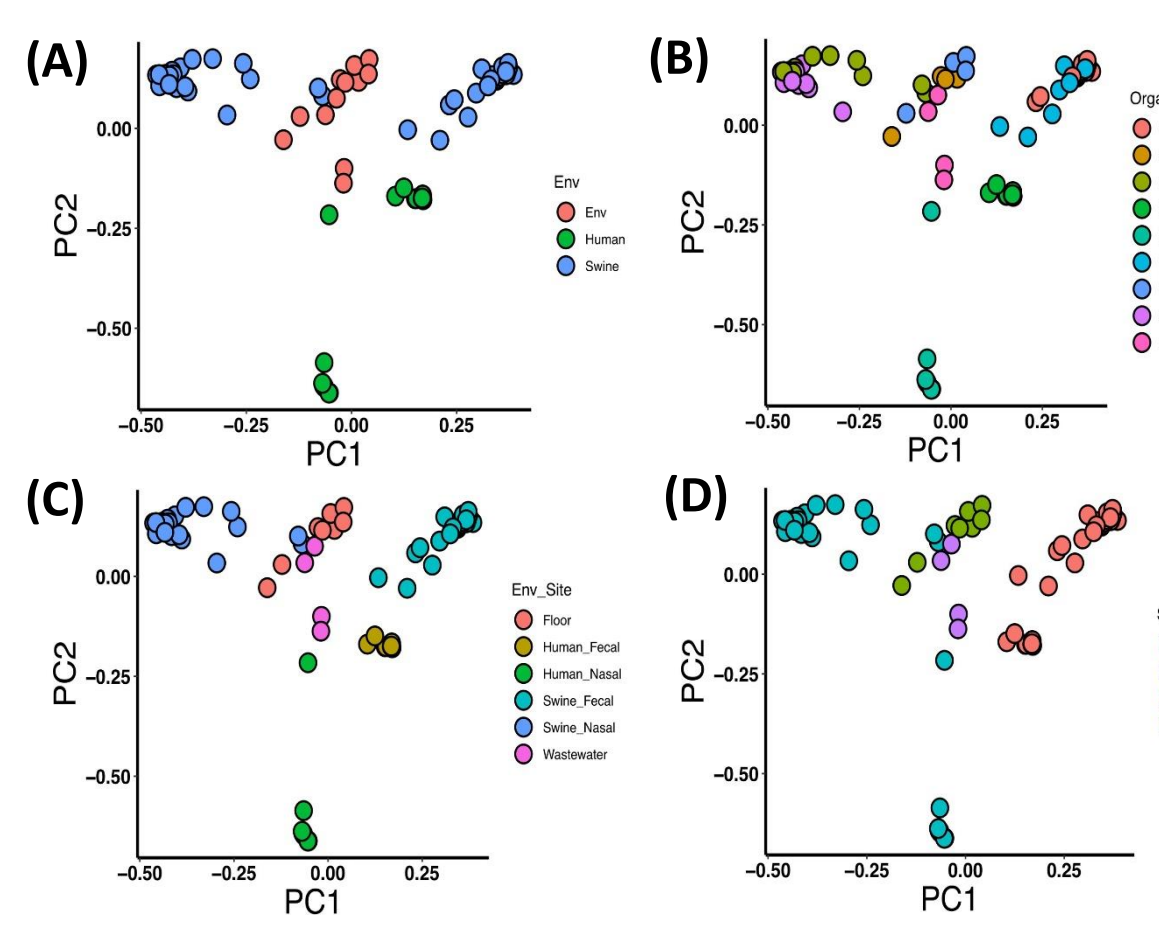


Fig 2. Dissimilarity of bacterial community
(A) The principal coordination analysis (PCoA) of Bray distance of bacterial community according to environment, (B) organism, (C) environment site, and (D) site. The bacterial community were different according to environments(P = 0.01), organism(P = 0.016), environment site(P = 0.01), and site(P = 0.05).

Antibiotic resistance gene class distribution

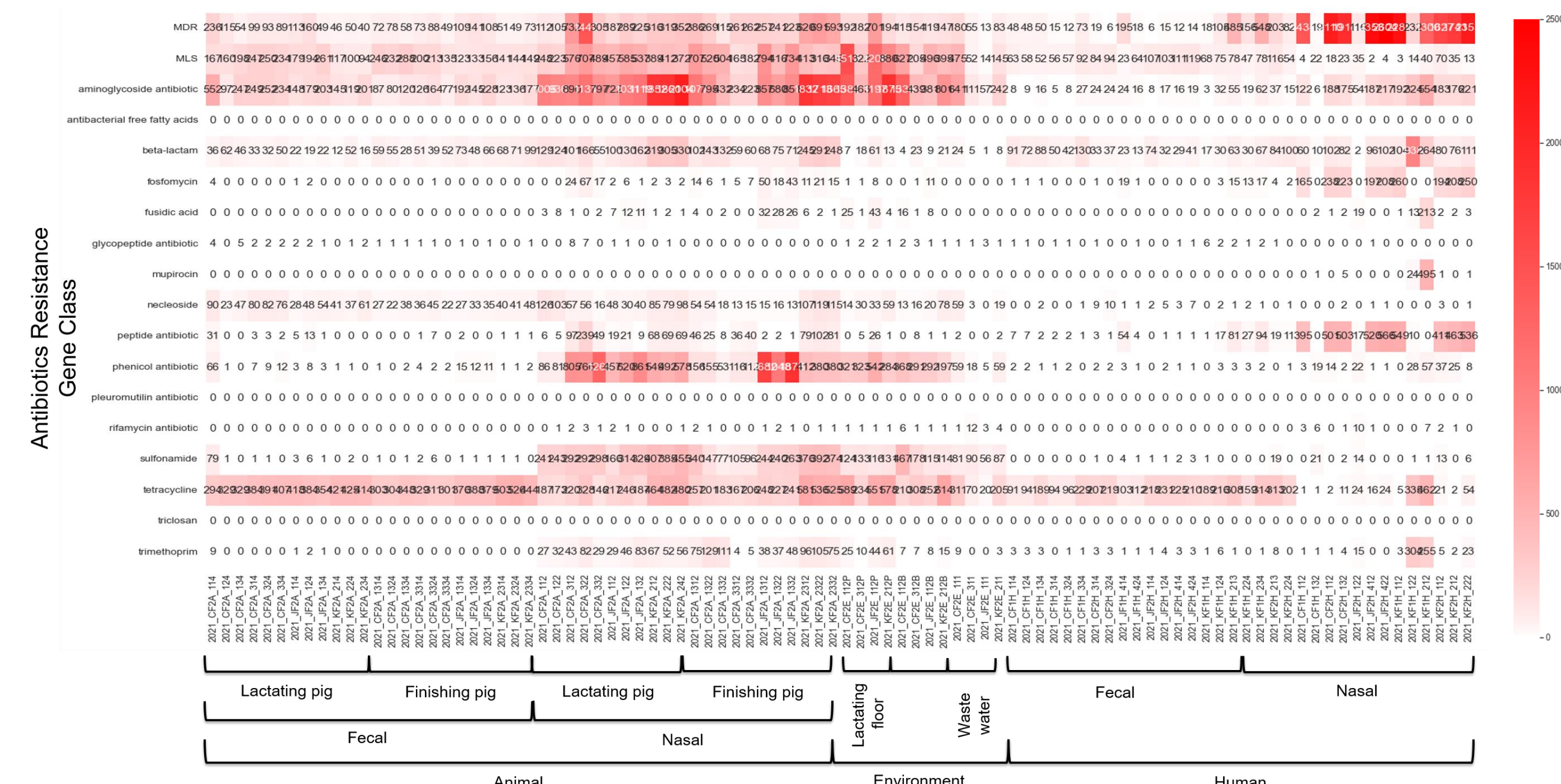


Fig 3. ARG class's relative abundance

- Aminoglycoside resistance gene is the most common in swine and environment samples. MDR(Multi-Drug Resistance) gene is the most common in human samples.
- Except for tetracycline resistance gene, the ARGs in the nasal samples tend to be higher than those in the fecal samples. MDR, MLS, and tetracycline resistance genes are common in all samples

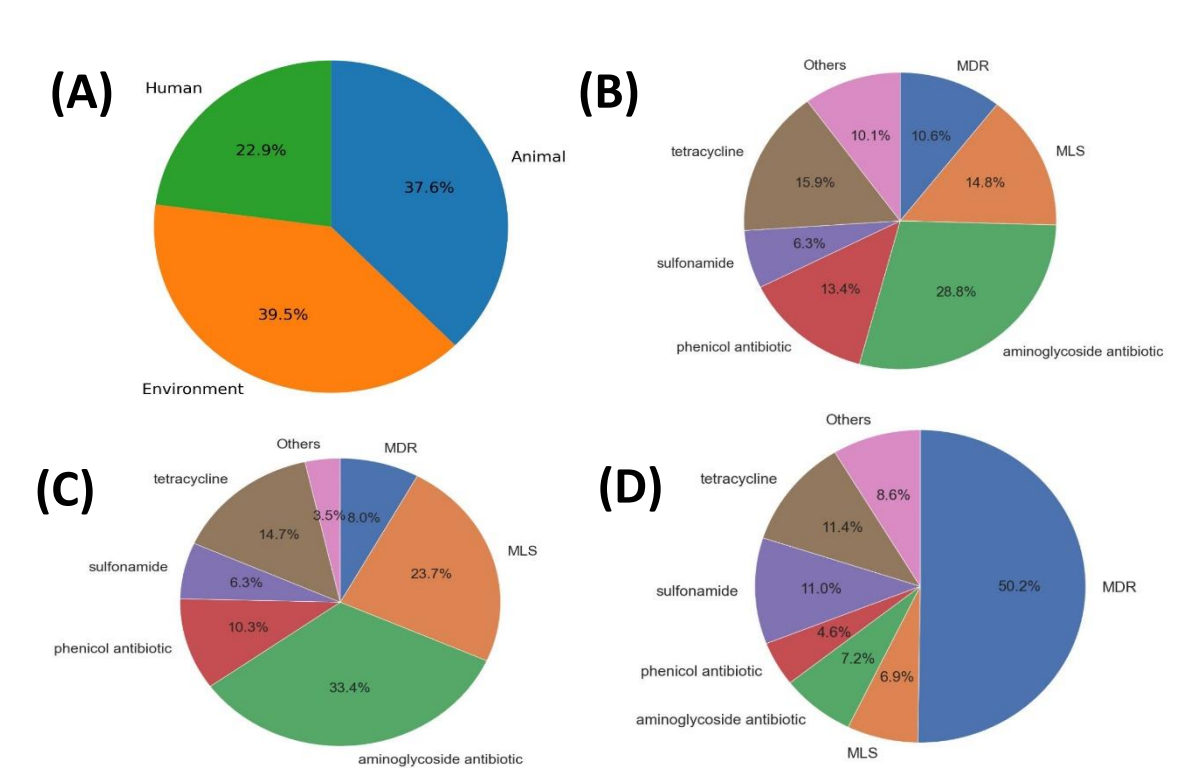


Fig 4. ARG class ratio

(A) Averaged ARG ratio grouping by sample's environment. The environment sample's averaged ARG ratio is the highest. (B) The Proportion of the classes according to swine samples, (C) environment samples, and (D) human samples. Resistance genes to Beta-lactam, fosfomycin, and peptide are high concentrations only in human samples. While resistance genes to phenicol and sulfonamide are high concentrations only in swine and environment samples.

Antibiotic resistance gene composition

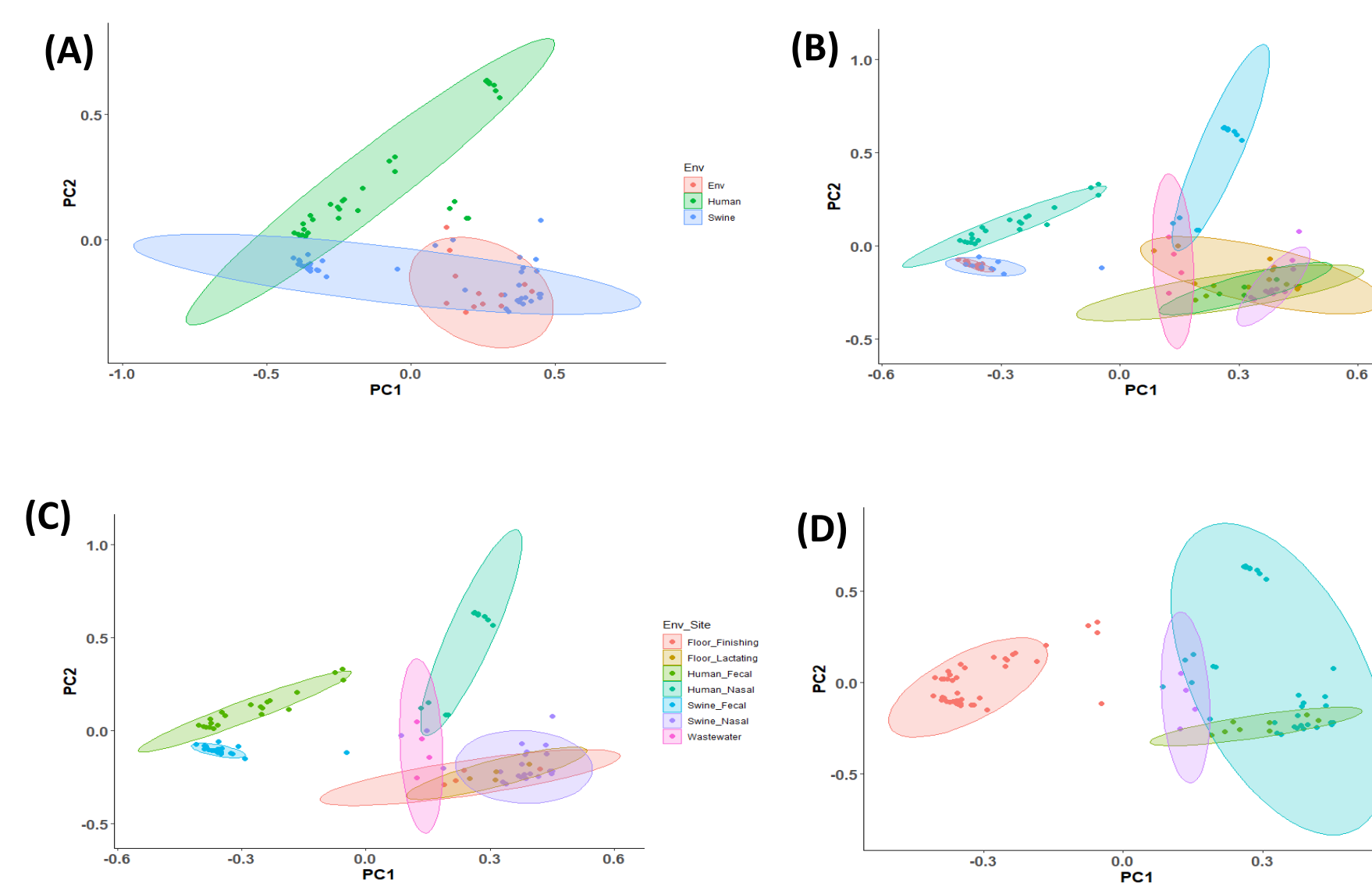


Fig 5. ARG PCoA

(A) The principal coordination analysis (PCoA) of Bray distance of ARG composition according to environment, (B) organism, (C) environment site, and (D) site. In all cases (A), (B), (C), and (D), the ARG composition is significantly different(P = 0.001). Furthermore, swine-environment is significantly different(P = 0.002), but there is no significant difference between human-swine(P = 0.536) and human-environment(P = 0.425).

Antibiotic resistance gene correlation analysis

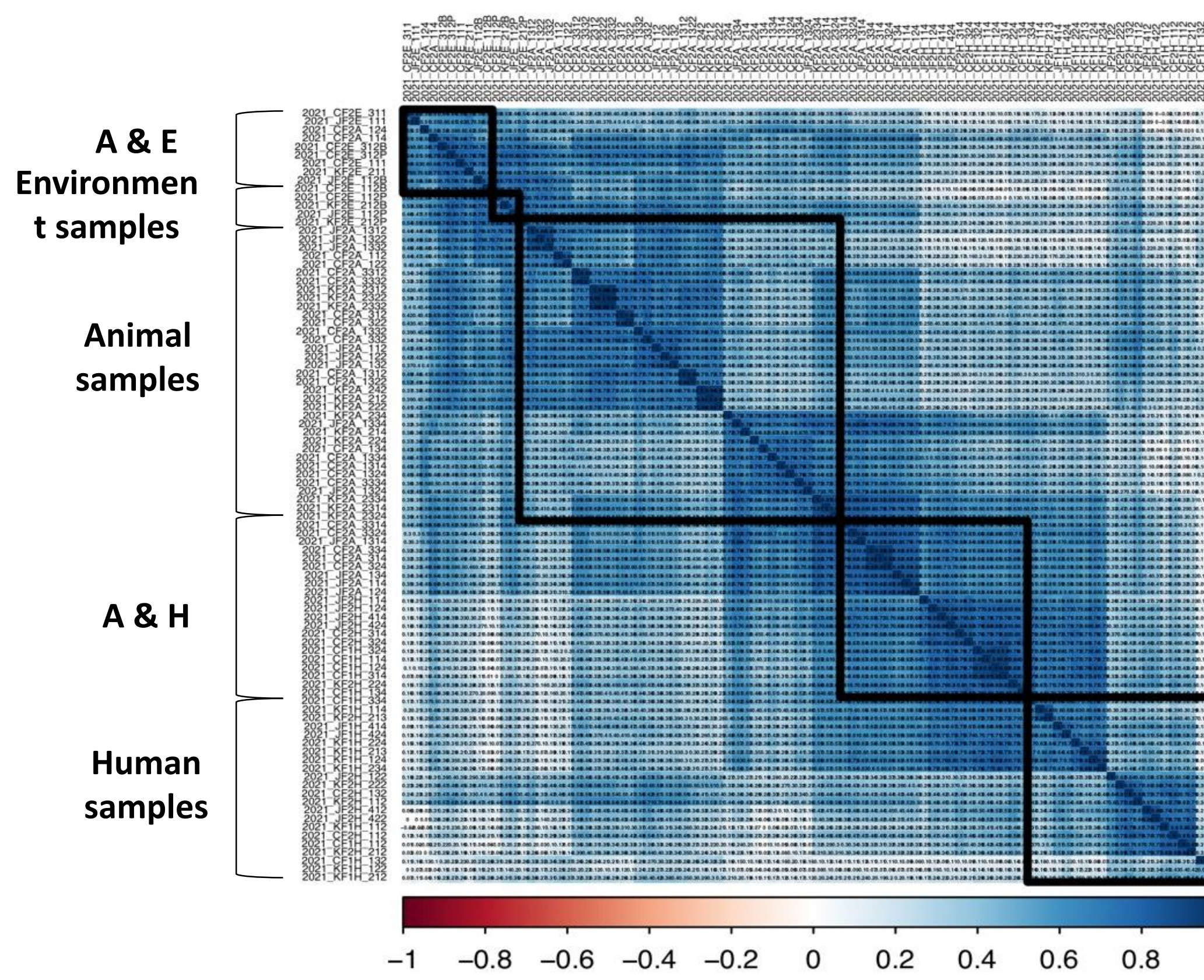


Fig 6. ARG correlation plot

- The Correlation of each gene in the sample was shown. All regions are blue, which means that the genes in the sample are positively correlated.
- Randomly list the samples and sort them using "hclust" (hierarchical clustering order) and draw 5 squares based on the results of hierarchical clustering. Thus, samples grouped in squares have similar correlations with each other. As a result, some animal & environment samples and animal & human samples have similar correlation coefficients.

Conclusions

The most abundant microbial phylum in the livestock farm sample was Bacteroidota. Bacterial community and ARG were differentiated according to environment, organism, environment site, and site. But, ARG composition was not differentiated depending on human-swine, human-environment. The most abundant ARG class in the livestock farm sample was aminoglycoside resistance. The distribution of ARG is similar between swine and environment, but there is a slight difference in human. The ARG of the sample has a positive correlation with each other. Human, swine, and environment have shared antibiotic resistance genes, and there is a possibility of propagation of resistance genes.

References

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- Mackenzie, John S., and Martyn Jeggo. "The One Health approach—Why is it so important?." *Tropical Medicine and Infectious Disease* 4.2 (2019): 88.