DISSERTATION

# ANTIMICROBIAL RESISTANCE IN THE MEAT INDUSTRY AND THE IMPACT OF MEAT ANIMAL FECAL MICROBIOMES AND RESISTOMES ON SUBSEQUENT ENVIRONMENTS

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ABSTRACT

ANTIMICROBIAL RESISTANCE IN THE MEAT INDUSTRY AND THE IMPACT OF MEAT ANIMAL FECAL MICROBIOMES AND RESISTOMES ON SUBSEQUENT ENVIRONMENTS

The discovery of antibiotics for human and animal use is considered one of the greatest medical advancements. However, the widespread use of antibiotics has caused concerns about a potential increase of antimicrobial resistance genes (ARGs) within microbial communities offering the opportunity for consumers to acquire antimicrobial resistant infections through the direct consumption of meat animals or through the environment via manure applications to crop land. Many consumers are becoming increasingly conscious of antibiotic usage labeling when purchasing meat products resulting in animal agriculture being considered a primary contributor for the dissemination of antimicrobial resistance (AMR). Although in recent years many advancements have been made to more fully understand the resistome of production animals preharvest and few post-harvest but, there are minimal studies that fully characterize the resistome of meat animals carcasses throughout the harvest process. Therefore, the purpose of the review (Chapter 2) is to outline opportunities to utilize metagenomic sequencing to pinpoint potential sources of antimicrobial resistance throughout meat processing. This could provide insight to better understand the potential sources of antibiotic resistant bacteria as a result of meat production.

As bacteria can acquire ARGs through horizontal gene transfer or mutations, as an evolutionary advantage directly resulting from environmental pressures, the objective of the following study (Chapter 3) was to evaluate the relationships between the fecal resistome of

different food animal species (avian, bovine, and porcine), the resistomes of meat from those animals, and resistomes of soil where feces was used as an amendment. Composite fecal samples ($n$ = 20 per species) were collected from each commercial production facility and meat rinsate samples were ($n$ = 20 per species) collected for each species at the time of harvest. After harvest, feces and litter were composted and applied as an amendment on agricultural land. After one growth season, soil samples ($n$ = 20 per species) were collected separately for each species. Additionally, human waste solids were collected from wastewater treatment plants near each animal production operation ($n$ = 14 per species), and soil samples amended with human waste solids were collected ($n$ = 7 per species) from fields in close proximity to the broiler and bovine facilities. DNA was extracted, and the resistome library was prepped using the SureSelectXT reagent kit to prepare samples for target-enriched resistome sequencing targeting ARGs. Reads were analyzed using AMR++ v2 pipeline and sequences were aligned to the MEGARes v2 database to identify ARGs. Richness, evenness, and Shannon's diversity were calculated, and beta-diversity was analyzed using Bray-Curtis dissimilarity distances. Hierarchal clustering was performed using Ward's agglomeration in R. Regardless of species, fecal samples had a greater ($P$ < 0.05) richness and evenness of ARGs compared to both meat and soil samples. For beta diversity, all the sampling types clustered ($P$ < 0.05) individually (feces, meat, and soil) by species. Furthermore, within species each environment was dominated by different classes of ARGs indicating they have different resistomes. When resistance groups medically important for human health by the World Health organization were considered, human waste samples had a greater ($P$ < 0.05) percentage (13%) of medically important resistance groups compared to all animal fecal samples (< 5%) (World Health Organization, 2017). The resistome of feces was richer and more diverse and clustered independently from both meat and soil indicating feces had a more unique

resistome across the different species. This suggests that the fecal resistome may not influence meat and amended soil resistomes.

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# DEDICATION

I would like to dedicate this dissertation to my parents Nicholas and Margaret Rice who have shown me endless love and encouragement throughout this entire journey. Without you both none of this would have been possible.

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## Antimicrobial Resistance

### *Antibiotic usage and resistance*

The discovery of antibiotics in 1928 by Alexander Fleming is by far one of the most important advancements in medicine. In a short amount of time, antibiotics were widely being utilized in both human medicine as well as agriculture. The usage of antibiotics has directly resulted in an increase in the average human lifespan due to the ability to treat infections that would have otherwise been fatal prior to antibiotic usage (Nicolaou and Rigol, 2018). Shortly after antibiotics became widely available for human use, the agriculture industry began utilizing antibiotics as therapeutic drugs for food production animals in an effort to prevent disease and supply the growing demand for food with high quality products that result from healthy herds of animals (Hardy, 2002). While the main goal of antibiotic use in livestock was to increase overall heard and flock health, it was discovered that antibiotic usage also improved growth and became a standard for growth promotion (Bacanlı and Başaran, 2019). Therefore, antibiotics not only elevated human medicine but increased animal health and efficiency.

Although large scale usage of antibiotics is a rather recent advancement in human history, antibiotic resistance is ancient. The ancient origins of AMR were outlined in a study by D'Costa et al. (2011), were they used a targeted metagenomic analyses on 30,000 year old permafrost samples and were able to identify a highly diverse collection of ARGs encoding for resistance to β-lactams, tetracyclines, and glycopeptides as well as, vancomycin resistance element (VanA) and they were able to confirm its similarity to modern variants.

In addition, ARGs were found in the gut contents of 1,000-2,000-year-old humans (Wibowo et al., 2021). Brealey et al. (2021) reported finding ARGs in the oral microbiota of Swedish bears collected over the last 180 years, well before the widespread use of antibiotics. All of these environments are considered pristine as they predate modern clinical use of antibiotic medications, and all of these studies provided researchers with evidence that antimicrobial resistance is naturally occurring. Although AMR is naturally occurring, the ability to develop resistance is a survival mechanism for bacteria giving them a competitive edge for survival against environmental pressures such as naturally occurring antibiotics produced by other bacteria (Bhullar et al., 2012). Although, ARGs naturally occurring, they have the ability to dissimilate throughout environments and spread to other bacteria via horizontal gene transfer. Horizontal gene transfer is the ability for bacteria to pass on ARGs to other species of bacteria. This can happen through three different mechanisms: conjugation, transformation, and transduction. Conjugation is a method of horizontal gene transfer where cells directly transfer genetic material from one another through direct cell to cell contact (Smillie et al., 2010). When utilizing transformation, bacteria uptake single strands of foreign genetic material and the recipient cell incorporates those genes into their own genomes for utilization (Johnston et al., 2014). Additionally, transduction is the sharing of genetic material from cell to cell utilizing phages (Brown-Jaque et al., 2015). It is through these mechanisms that bacteria are able to quickly evolve and develop resistance to common or misused antibiotics.

*Dissemination of antimicrobial resistance*

Recently, the world has given more attention to antibiotic usage especially across animal agriculture. The World Health Organization has acknowledged AMR as a global public health threat and outlined several potential source for the transmission of ARGs to humans (World Health Organization, 2019). Transmission of ARGs to humans has been outlined to occur through

multiple different pathways. Landers et al. (2012) outlined multiple potential pathways for the transmission of ARGs to humans. The major pathways outlined as potential vectors for the dissemination of ARGs to humans from animal agriculture are through direct contact with animals, through the environment, and some speculate through the consumption of meat products (Mayrhofer et al., 2004; Leverstein-van Hall et al., 2011; Marshall and Levy, 2011). Manure from livestock both treated with antibiotics and antibiotic free are both possible sources of environmental dissemination of ARGs into the environment through horizontal gene transfer (Holmes et al., 2016; von Wintersdorff et al., 2016).

In addition to ARG transmission from animals to humans, human waste is also a reservoir for medically important ARGs (Harris et al., 2014). In a study by Harris et al. (2014), they wanted to determine the extent that human effluent from hospitals contributed to the dissemination of ARGs into the environment by monitoring two waste-water treatment plants. One plant received effluent from a hospital while one did not, and they monitored the waste-water treatment plants for antimicrobial resistance of *Escherichia coli.* They reported there was no statistical difference in resistant *Escherichia coli,* between the waste-water treatment plants, and ultimately determined that hospitals alone were not the sole source of resistant *Escherichia coli* in human wastewater. With the presence of resistance in human waste, there is potential for the transmission of ARGs medically important for human health to be transmitted from humans to animals. A study by Ma et al. (2016), explored the ARGs and the shared fecal resistome of swine, chickens and humans. They reported finding high levels of tetracycline, multidrug, erythromycin, and aminoglycoside resistance genes across all the fecal samples from all the species. Additionally, there were many shared ARGs between the species and mobile the genetic elements aadA5-dfrA17-carrying class 1 integron were identified in chicken feces and were carried by human pathogens.

Antibiotics are utilized in agriculture as a method to combat the growing demand for animal protein. Antibiotics can be used for meat production animals as a way to treat and prevent diseases and potential subclinical symptoms, and in sub-therapeutic levels as a form of growth promotion (Hao et al., 2014; You and Silbergeld, 2014). It is thought that the intensive use of antibiotics in food production animals can lead to the emergence of resistance due to misuse.

*Antimicrobial usage and resistance in poultry*

Chicken is the fastest growing protein groups in the United States with the value of broiler produced during 2020 exceeding $21 billion (USDA ERS - U.S. Per Capita Availability of Red Meat, Poultry, and Seafood on the Rise). With the continued growth of the broiler industry, antibiotics are typically administered to entire flocks for the treatment of diseases, disease prevention, and as a growth promotant (Poole and Sheffield, 2013). In the United States, there has been a significant decrease in the use of antibiotics in poultry. In 2013, 93% of broilers were receiving antibiotics during production and by 2017 that percentage had dropped drastically to 17%. This decrease can also be represented as a 74% decrease in the use of gentamicin in hatcheries, a 95% decrease in tetracyclines and 60% decrease in virginamycin in broiler feed, and a 21% decrease in penicillin, 47% decrease in tetracyclines, and 72% decrease in sulfonamides as water soluble antibiotics in broilers (Singer et al., 2020).

Even with the decrease in antibiotic usage, antibiotic resistance is still a major concern in the United States. Hedman et al. (2020) reported high levels of *Campylobacter* resistance to ciprofloxacin in humas was associated to poultry consumption and direct contact with animals. Additionally, they observed moderate levels of *Salmonella* resistant to ciprofloxacin which they associated with direct or indirect contact with animal feces. Further, multi-drug resistant *Salmonella enterica* serotype *Infantis* recovered from broilers was revealed in sick people

returning from South America (Hedman et al., 2020). Therefore, two important pathways for potential dissemination of resistance in poultry are feces and meat consumption. De Cesare et al. (2022) performed a pilot study in the European Union where they characterized the caeca and corresponding carcasses of chickens raised conventionally, and chickens raised without antibiotics to determine if there was any antimicrobial resistance gene spread pre or post-harvest. They reported clear separation in the caeca samples between conventional and antibiotic free rearing for antimicrobial resistance, however they did not find any differences in the carcass resistome between the two production practices. They also noted that there was a greater antimicrobial resistance load on carcasses compared to the caeca and concluded that all post-harvest steps contribute to the resistance load on the final carcass that reaches consumers. In another study by Li et al. (2020) where they analyzed the resistome of retail chicken breasts, they also found that production practice (conventional vs antibiotic free) did affect the resistome of the chicken breast and that a majority of the 10 ARGs belonged to aminoglycosides and ß-lactams. They also determined that the packaging type of the chicken breasts impacted the resistome. and found a low abundance of ARGs on chicken breasts compared to De Cesare et al. (2022) which they attributed to the regulatory differences between the United States and the European Union. While the earlier study by Li et al. (2020) ultimately concluded there was minimal risk of dissemination of ARGs to humans through the consumption of chicken breast regardless of production method. However, the more recent paper by De Cesare et al. (2022) speculated that different parts of the harvest process all contribute to the ARGs on the chicken carcasses, and more research needs to be done at those different steps in the process.

*Antimicrobial usage and resistance in beef cattle*

Antimicrobial usage in beef production is used for similar purposes as mentioned previously in the poultry industry. In the cattle production antibiotic usage includes aminoglycosides, β-lactams, chloramphenicol, fluoroquinolones, glycolipids, ionophores, macrolides, quinolones, streptogramins, sulfonamides, and tetracyclines. One of the key time points of antibiotic usage in beef cattle is during the transition to a feedlot setting. This transition can be extremely stressful for animals as they are experiencing new handling practices, being transported, and commingling with other cattle which all can make the cattle susceptible to illnesses such as bovine respiratory disease (Sanderson et al., 2008). Because of this, cattle are typically treated with antibiotics upon entry into the feedlot to prevent subclinical symptoms from becoming clinical and preventing death (metaphylaxis). Doster et al. (2018), examined the impact of metaphylactic treatment of beef cattle with tulathromycin on the fecal resistome using shotgun metagenomics. They reported that the resistome of fecal samples collected from treated and untreated animals were similar at both day 1 and day 11. Since animal feces is considered a primary vector for the dissemination of antimicrobial resistant bacteria in the environment when manure is used as an application to soil for crops, this could indicate a similar potential of dissemination of ARGs in the early feeding period. However, in a study comparing fecal ARGs of dairy cattle treated with third generation cephalosporins, the researchers reported that antibiotic treatment of beef cattle increased the ARGs present in feces, in comparison to feces of untreated cattle, conversely indicating cattle treated with antibiotics have an increased risk of resistant bacteria (Chambers et al., 2015).

In addition to the fear of dissemination of ARGs through feces, ARG dissemination through consumption of beef products is also a concern. Although many different procedures are

in place to prevent antibiotic residues from entering the food supply, many beef products carry label claims such as “antibiotic free” or “raised without antibiotics”. Several studies have investigated the resistome of beef products from both conventional and raised without antibiotics production styles. In a study by Doster et al. (2020b), they used target enriched metagenomics to compare ground beef from local grocery stores that were labeled as raised without antibiotics, and conventionally raised. They determined that there was no difference in the resistome of ground beef regardless of the label. In a study by Schmidt et al. (2020) they used utilized microbial culture based methods for the detection of five antimicrobials, from conventionally and raised without antibiotics ground beef samples from six different cities in the United States. They reported that the prevalence of tetracycline-resistant *Escherichia coli* (conventional, 46.3%; raised without antibiotics, 34.4%) and erythromycin-resistant *Enterococcus* (conventional, 48.0%; raised without antibiotics, 37.5%) was higher in in ground beef not labeled as raised without antibiotics. Additionally, the ARG blaCTX-M was more abundant in conventionally raised ground beef, while the ARGs mecA, tet(A), tet(B), and tet(M) were more abundant in ground beef ground beef labeled as raised without antibiotics, which should be lower since the animals were not treated with tetracycline drugs. Ultimately, they concluded antibiotic usage did not increase the risk of antimicrobial resistance in humans. Weinroth et al. (2022), compared the colon resistome and the resistome of meat trimmings of cull beef and dairy cows from conventional and raised without antibiotic production practices. They reported that tetracyclines were the most abundant ARG class for both the colon content and meat trimmings. Additionally, they reported differences in ARGs in colon content based on production practice, but there were no differences in resistome of the meat trimmings based on production practice, ultimately determining that there is minimal risk of

dissemination of ARGs to human through the consumption of meat form conventionally raised animals.

*Antimicrobial usage and resistance in swine*

Just as with antibiotic usage in both poultry and beef cattle, antibiotics are utilized by the swine industry to treat disease and are delivered in low doses through both water and feed to increase performance by increasing rate of gain and feed efficiency (Cromwell, 2002). More recently, similarly to poultry, there has been a shift in the swine industry to focus on sustainability resulting in a shift away from antimicrobial usage (Zeineldin et al., 2019).

Even with this focus on sustainability, AMR transmission from swine to human is still a concern in the industry. Kazimierczak et al. (2009) examined how the discontinuation of antibiotic use would affect ARGs in pigs by conducting a metagenomic analyses of pigs reared in an antibiotic free environment to determine their resistome. They subsequently sequenced ARGs and specifically noted this was the first time that tet(40) had been found outside the human gut. In a later study, Zwonitzer et al. (2016) sampled six different swine operations consisting of two antibiotic free and four conventional farms where antibiotics are fed prophylactically. They reported that isolates from conventional systems were significantly more resistant to amoxicillin, ampicillin, chlortetracycline, erythromycin, kanamycin, neomycin, streptomycin, tetracycline, and tylosin, but also found resistant *Escherichia coli* in isolates from organic manure. Lugsomya et al. (2018) also compared conventional and raised without antibiotics production methods and found that *Escherichia coli* resistant to aminoglycosides and extended-spectrum ß-lactams (ESBL) were greater in conventional swine farms. Similarly, Thakur and Gebreyes (2005) showed antimicrobial resistant Campylobacter was present in the manure of pigs raised both conventionally, and without antibiotics. In a study that assessed the subtherapeutic administration of tylosin and

chlortetracycline on the AMR of swine they reported that pigs supplemented with tylosin had a significantly greater prevalence of tylosin resistant bacteria compared to non-supplemented pigs, and the ARGs tet(O), tet(Q), and erm(B) were detected in all pigs (Holman and Chénier, 2013). Due to the application of swine manure to crop land as a soil amendment it is important to fully understand how ARGs move through the environment.

Very few studies have taken a look into the resistome of pork post-harvest. Vikram et al. (2019) collected pork chops from animals raised without antibiotics and conventionally from food service suppliers and cultured Escherichia coli, tetracycline-resistant *Escherichia coli*, third-generation cephalosporin-resistant *Escherichia coli*, *Salmonella enterica*, tetracycline-resistant *Salmonella*, third-generation cephalosporin-resistant *Salmonella*, nalidixic acid–resistant *Salmonella*, *Enterococcus* spp., tetracycline-resistant *Enterococcus*, erythromycin-resistant *Enterococcus*, *Staphylococcus aureus*, and methicillin-resistant *Staphylococcus aureus*. They determined that production practice did not impact the detection of cultured bacteria. Additionally, quantitative PCR was used to assess the aac(6′)-Ie-aph(2″)-Ia, aadA1, blaCMY-2, blaCTX-M, blaKPC-2, erm(B), mecA, tet(A), tet(B), and tet(M), and there were no differences between the swine production practices. Some of the research has also focused on the harvest facility. A study by Liu et al. (2020) focused solely on resistance in salmonella in pork production systems in China. They isolated 105 *Salmonella* strains from pig carcasses and the environment of 4 separate harvest facilities. They reported that all isolates were multi-drug resistant with 95% being resistant to tetracyclines and 91% resistant to trimethoprim and sulfamethoxazole. Cobo-Díaz et al. (2021) recorded the establishment of the microbial environment and resistome in the first 1.5 years of a newly built pork fabrication facility in Spain. They took samples of pork carcasses as well as environmental samples of the plant including belts, drains, and equipment. They noted the

resistome structure temporally evolved, and Acinetobacter and Pseudomonas were the most relevant carriers of ARGs and the most abundant ARGs associated with resistance were to betalactams (27.2%), tetracyclines (26.9%), aminoglycosides (25.3%), and quinolones (7.5%.). However, over time, there was significant increase an in the relative abundance of ARGs related to resistance to aminoglycosides, tetracyclines, and antimicrobials of the MLSP group (macrolides, lincosamides, streptogramins, and pleuromutilins). They also noted that mobile genetic elements were greater in drains and increased over time. They ultimately concluded that the pork carcasses themselves were a primary source of ARGs in the new facility.

*Antimicrobial resistance in soil and composted manure*

Another opportunity for ARGs to reach humans is through the application of manure as a fertilizer to crop land as animal manure is a rich source of ARGs regardless of whether or not those animals were treated with antibiotics (Topp et al., 2018). In a study by Chen et al. (2019) they compared the antimicrobial resistance gene profiles amongst soils with different manure amendment applications 120 days after application they reported the environmental resistome risk scores of all amended soils including manure from animals treated with antibiotics were equivalent to that of the unamended soils suggesting the soil was able to recover from the amendments. However, Macedo et al. (2020) tested soil with different textures to determine its ability to recover its beginning levels of ARG after manure application. They determined that manure application increased *erm(*B) and *tet*(W) resistance in the soil, and the type of soil impacted its ability to recover at a certain rate. They predicted that soil final recover times occurred between 29 – 42 days, which is significantly shorter than the 120 days out lined in Chen et al. (2019). Further, Joy et al. (2013) tested the ARGs in soil and runoff following land application of swine manure slurries and determined the land application method did not impact the concentration of ARGs in

subsequent run off and the concentration of ARGs (chlortetracycline and tylosin) decreased with each rainfall event. In another study to examine the ARG load in swine manure slurries, Sanz et al. (2021) found ARGs representative of those found in animal feces and the levels of some antibiotics exceeded currently accepted minimum inhibitory concentrations. Sanz et al. (2021) additionally, found evidence that ARG loads were directly correlated to the prevalence of *Actinobacteria*, *Proteobacteria*, and *Spirochaeta*. H. Yang et al. (2020), used a microbial culture confirmation method and qPCR to test ARG prevalence in soil due to the amending with manure as a long-term pasture management strategy. They determined that long term use of swine manure as a soil amendment could increase the abundance of ARGs in the soil. However, Miller et al. (2019) determined cropland amendment with manure from beef cattle has a minimal impact on ARGs in soil. Ultimately, most of the forementioned studies were in agreement that although there was an initial increase in ARGs after manure application, with time, the resistome of soil was able to recover resulting in less ARGs present.

*Approaches to investigate antimicrobial resistance*

Until recently, all work in antimicrobial resistance was performed using culture confirmation. As technology improved, antimicrobial resistance has shifted from culture-confirmation to the use of qPCR, and most recently utilizing shotgun sequencing to determine the whole resistome of an environment. Three major studies utilizing the same samples provided key insights to the benefits and disadvantages of each method (Kanwar et al., 2013; Kanwar et al., 2014; Weinroth et al., 2018). In the first study, Kanwar et al. (2013) utilized a broth microdilution to isolate non type specific *Escherichia coli*, and then used qPCR assays to specifically detect tet(A), tet(B) and blacmy-2. They concluded that chlortetracycline treatment resulted in an increased probability of recovering ceftiofur resistant isolates at the phenotypic and genotypic

level and chlortetracycline appeared to increase ceftiofur resistance levels following ceftiofur crystalline free acid treatment. In the second study, Kanwar et al. (2014) extracted DNA directly from the feces and utilized qPCR with primers to target the genes: blacym-2, blactx-m, tet(A), tet(B) and 16S rRNA. They concluded that chlortetracycline should not be used in an attempt to control ceftiofur-resistance following treatment in with a cephalosporin. Chlortetracycline treatment led to an increase in both blacmy-2 and blactx-m gene copies, especially in pens of cattle where all the animals were exposed to ceftiofur. Pen level ceftiofur resistance was lower in pens were just one ceftiofur crystalline free acid treated animal was cohoused with non-treated pen mates. In the third study, Weinroth et al. (2018) analyzed the same samples using shotgun metagenomics. They concluded there were no significant detectable changes in the relative abundances of betalactam ARGs in the feces of cattle in association with treatment of with ceftiofur crystalline free acid although diversity was impacted. The relative abundances of tetracycline ARGs increased on day 26 after exposure of the cattle to chlortetracycline via feed. There was also an increased abundance of aminoglycoside-resistant genes at the end of the trial in all cattle. Because of the different methods, the results from the culture and qPCR studies did not mimic the results represented in the shotgun sequencing study even though the samples were the same. This could be expected due to the differences in the methods used. Both culture and qPCR are very targeted methods for the identification of antimicrobial resistance genes. In the first study where culture and PCR methods were used, they were able to only identify the assay specific genes in only the *Escherichia coli* samples they had selected for during the culture step. In the second study where they only used qPCR, they were able to be slightly less selective, but they were only able to determine resistance based on the gene specific primers they used during amplification. Essentially, they selected for only blacym-2, blactx-m, tet(A), and tet(B), and that is what was

amplified during PCR. The inability to identify sequences in shotgun that were identified in PCR was expected. During shotgun sequencing, all genetic material is amplified. This means that less prevalent taxa may not be as detectable compared to taxa that are present in higher quantities. As they were highly selected, these taxa were able to be detected in the culture and PCR methods.

Both qPCR and shotgun sequencing are DNA amplification methods, but they resulted different information. Results from qPCR are very selective, as specific primers are used to target predetermined genes allowing them to be detected and quantified. Shotgun sequencing amplifies all DNA (bacterial, viral, and eurkaryotic) in the sample providing a more complete picture of the microbial community. While shotgun sequencing can provide more detailed taxonomic resolution that can provide information regarding functional properties, less prevalent taxa can be lost during amplification. In the previously mentioned studies, this is evident as some of the genes that were targeted for during culture and qPCR did not show up in the shotgun analysis. This could be because they were less prevalent. The method of investigation into ARGs within an environment depends solely on the research question.

*Target Enrichment*

Although metagenomic sequencing is useful in the research of antimicrobial resistance genes, samples with greater amounts of background host DNA should be addressed with caution. Samples such as meat rinsates contain a greater amount of host DNA compared to environmental samples. Noyes et al. (2017) utilized a method on "on target" sequencing where a specific bait system was used to select for, or target enrich the samples for antimicrobial resistance. This enrichment allows for the identification of rare resistance genes that would otherwise not be detected. With the utilization of baits, there is less opportunity for host DNA to contaminate the samples during sequencing. This allows for researchers to focus on specific genes within samples

ultimately reducing cost and easing the later analyses. Using a hybridization method, specially designed baits that contain probes complementary to the targeted regions are bound to DNA molecules and then purified using magnetic beads to prevent the targeted genes from being rinsed away. This leaves only the targeted regions for downstream analyses (Bodi et al., 2013).

# CHAPTER 2 - A REVIEW OF THE PREVALENCE OF ANTIMICROBIAL RESISTANCE IN MEAT ANIMALS DURING PROCESSING AND IN A RETAIL STORE

## Introduction

The increase in awareness of the potential health threat from antimicrobial resistant bacteria have caused growing consumer concern in regard to meat animal production and the consumption of meat and potential vectors for the transmission of antimicrobial resistant bacteria to humans. Antimicrobial drug use in meat animals and subsequent antimicrobial resistance (AMR) transmission is quickly becoming a major concern for meat consumers around the world. The development and implementation of antimicrobial drug treatment in production animals has been a major milestone in animal health improving herd and flock health. Antibiotic drug usage in livestock provides benefits to food animal health and welfare, by therapeutically treating infections that can potentially spread throughout large production systems causing increased mortality rates and lower rates of gain. Additionally, antibiotic drugs can be administered preventatively to stop any potential infections that could be subclinical.

The World Health Organization (WHO, 2015; WHO, 2017; WHO, 2019) and Centers for Disease Control and Prevention (CDC, 2019; CDC, 2021) have labeled antimicrobial resistance as one of the most critical threats in public health. The Centers for Disease Control estimates each year 2.8 million antibiotic resistant infections occur in the United States, resulting in upwards of 35,000 deaths per year (CDC, 2019). Additionally, Nelson et al. (2021) reported just six multi drug resistant bacteria (methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococci*, extended-spectrum ß-lactamase, carbapenem-resistant *Enterobacteriaceae*, carbapenem-resistant *Acinetobacter*, and multidrug-resistant *Pseudomonas*) to have a potential economic impact of over $4.6 billion annually only adding to the overall impact of resistant bacteria.

Much of the previous AMR surveillance research has been done using culture dependent methods. With aerobic culturing, bacterial isolates are tested for resistance to various drugs (Giuliano et al., 2019). Although culture-based studies have provided a plethora of information about AMR in many different environments, these culture-based methods are limited to the less than two percent of bacteria that is actually culturable in a lab setting (Wade, 2002). Therefore, culture-based method can only provide a highly selective look into the resistance potential in a microbial community. Due to the potential for transfer of resistance genes between microorganisms through horizontal gene transfer and mobile genetic elements, resistance is not confined to a specific bacterial species and can be fluid within microbial communities (Ghosh et al., 2013; Martínez and Baquero, 2014; Brinkac et al., 2017). With all this considered, next-generation sequencing, offers the opportunity to comprehensively view all the resistance genes in a microbial community (resistome), without the limitation of our ability to culture a bacterial species in a lab. There are many studies that focus on the gut and fecal resistome of food production animals, but few studies have taken a deeper looking in to the resistome of meat animal carcasses throughout the harvest process, and into retail using next generation sequencing (NGS). Therefore, this review evaluated published studies that assessed antimicrobial resistance on carcasses in different meat processing facilities and in retail meat products to determine potential vectors of AMR resulting from the consumption of meat products.

**Materials and Methods**

Scientific articles were selected related to the research objective presented above. The literature was sourced from international online databases and was retrieved using a combination of the key search terms: “resistome, antibiotic resistance, metagenomics, avian, poultry, broiler,

chicken, swine, porcine, pork, bovine, cattle, beef, meat, carcass, processing plant." Only articles published over the period of January 2002 to April 2022 were considered in the present review.

## Review and Discussion

### *Antibiotic resistance development and dissemination*

Although antibiotic drugs and AMR is currently a major topic in public health, antibiotics have origins which are considerably older than the discovery of penicillin by Sir Alexander Fleming in the early 1900's. Naturally occurring antibiotics have evolved over millions of years, and subsequently antibiotic resistance is an equally natural occurring phenomenon with resistance genes being identified in samples of frozen permafrost samples that were dated to be over 30,000 years old, and in the gut contents of 2,000 year old human remains (D'Costa et al., 2011; Wibowo et al., 2021). Many of the antibiotic mechanisms we currently utilize in modern medicine and in agriculture most likely originated from genes in environmental bacteria which have existed for thousands of years. Although these antibiotic and resistance genes in bacteria are natural, they both are survival mechanisms utilized by these organisms to provide an advantage during competition for resources. This competition for resources can pressure bacteria to form resistance to antibiotics through mutations occurring during cell replication or horizontal gene transfer which allows resistance to disseminate quickly to multiple species with selective pressures (Bhullar et al., 2012).

There are three different mechanisms in which cells can transfer genetic material to one another. The first mechanism of horizontal gene transfer is conjugation. Conjugation is a process in which cells transfer genetic material through direct cell to cell contact with surface pili or adhesins. Once the cells are coupled, genetic material in the form of plasmids are transferred from one cell to another by conjugative machinery within the cell (Smillie et al., 2010; Wozniak and Waldor, 2010; von Wintersdorff et al., 2016). Transformation is another mechanism of horizontal

gene transfer. Transformation, is the uptake of single strands of foreign DNA that is then integrated into the bacterial chromosomes of the recipient cell (Johnston et al., 2014). The DNA must be integrated into the recipient genome or circularized into a plasmid, and the resistance gene can then be utilized within the recipient cell and conserved. Lastly, resistance can be transferred via transduction. Transduction is the use of bacteriophages to transfer of advantageous genes to host bacteria and does not require cell to cell contact. These advantageous genes can be in the form of chromosomal DNA, plasmids, transposons, and genomic islands (Brown-Jaque et al., 2015). Through these mechanisms, bacteria are able to adapt to their environments and quickly develop resistance to heavily used antibiotics. This has caused antibiotic resistance to a public health crisis, with constant misuse of commonly used antibiotic medications in humans and animals, ultimately creating multi-drug resistant strains of bacteria which are resistant to three or more classes of antibiotic treatment.

*The use of antibiotics in animal production and harvest*

Animal agriculture is considered a key driver involved in the surge of antimicrobial resistance that is quickly becoming a public concern. Not long after the discovery of penicillin, antibiotic use in animal agriculture became a common occurrence and has been critical in the maintenance of herd and flock health and welfare as they vital in the prevention of and treatment of infectious diseases. Additionally, with improved herd and flock health, there was a subsequent improvement in feed efficiency. These advantages of antibiotic usage in livestock have resulted in the estimated sale of 60% of antibiotics sold in the United States in 2015 sold for utilization in food production animals (FDA, 2016). In the more current Annual Summary Report on Antimicrobials Sold or Distributed in 2020 for Use in Food-Producing Animals released by the United States Food and Drug administration (FDA, 2021), there was a 38% decrease in antibiotics

purchases for food production animals from 2015 to 2020, with a 3% decrease just from 2019 to 2020. They attribute this to ongoing efforts to support more judicious use of antibiotics in food producing animals. There are over 100 antibiotics currently utilized in livestock operations. Some of the more common classes include: ß-lactams, aminoglycosides, tetracyclines, cephalosporins, amphenicols, macrolides, sulfonamides, fluoroquinolones, lincosamides, polypeptides, and polyenes. The FDA also reported that there was a 4% decrease in the domestic sales of tetracyclines, which represent the largest portion of domestic sales in the United States (66%), from 2019 to 2020. In addition to tetracyclines, ß-lactams account for 13%, macrolides for 7%, sulfonamides for 5%, aminoglycosides for 5%, lincosamides for 2%, cephalosporins for less than 1%, and fluoroquinolones for less than 1% of the antimicrobials which are considered medically important classes in human medical therapy by the FDA sold in the United States for food producing animals. More specifically, an estimated 80% of cephalosporins, 57% of sulfonamides, 54% of aminoglycosides, and 43% of tetracyclines were intended for use in cattle. An estimated 87% of lincosamides and 42% of macrolides were intended for use in swine and an estimated 64% of ß-lactams were intended for use in turkeys. Additionally, antibiotics for cattle and swine accounted for an overwhelming majority of the medically important antimicrobial drugs sold from 2016 to 2020 making antibiotics a key factor in maintaining healthy and productive animals (FDA, 2021). Many of the concerns surrounding antibiotic usage in food production animals stems from the idea that the consumption of products derived from animals treated with antibiotic drugs could increase the risk of antibiotic resistant bacterial infections in humans that are increasingly more difficult to treat.

In addition to antibiotic treatment during animal production, bacteria are additionally subjected to antimicrobials during the harvest process for cattle, swine and poultry. Regardless of

species, sanitation is important to ensuring safe meat products, because inherently meat production animals harbor many different bacteria in their intestinal tracts, some of which pose a potential threat to human health. Some of the more notable foodborne pathogens associated with meat production include *Salmonella* spp., *Staphylococcus aureus, Escherichia coli, Campylobacter jejuni, Listeria monocytogenes, Clostridium perfringens,* and *Yersinia enterocolitica* which are all major public health concerns. During the harvest process many critical sanitation steps are taken to ensure the resulting meat products are pathogen free and safe for consumption. However, even with many different efforts in place to prevent foodborne pathogens in meat, the United States Centers for Disease Control and Prevention estimated that between 1998 and 2008, 22% of the foodborne related illness cases in the United States resulted from the consumption of meat and poultry, and the greatest percentage (29%) of the foodborne illness related deaths were attributed to meat and poultry products, while only 23% of the total deaths were shown to have come from produce (CDC, 2019). Most of the deaths associated with meat and poultry products can be attributed to poultry products. This makes it even more important many different steps are taken to prevent pathogens during both harvest and further processing of meat product. With this in mind, even ARGS in non-pathogenic bacteria should be of concern, due to bacteria's ability to acquire ARGs under pressure, and it can become a deathly combination when bacteria known to be pathogenic are able to acquire antimicrobial resistance mechanisms to both prevention antimicrobials and therapeutic antibiotics when they enter the food supply.

*Broiler*

Foodborne illness that can be traced back to poultry meat products has been estimated to cost over $2.4 billion per year in the United States, and *Salmonella* spp. and *Campylobacter* spp. the main pathogens of concern in the poultry industry (Lemonakis et al., 2017). In 2016, the United

States Department of Agriculture (USDA) agency Food Safety Inspection Service announced more stringent guidelines that required the prevalence of Salmonella positive not to exceed 9.8%, and the prevalence of Campylobacter positive carcasses not to exceed 15.7% of tested broiler chicken carcasses (USDA, 2022). In order for poultry processing facilities to meet these strict requirements, different antimicrobial processing aids are utilized. Typically, biocides, these antimicrobial processing aids are primarily used during the chilling process to reduce the prevalence of pathogenic bacteria to the acceptable levels dictated by the USDA, and ultimately improving the shelf life of those meat products. Some of the more common processing aids in the poultry industry are chlorine, peroxyacetic acid, and sodium hypochlorite. To date, few studies have used NGS to get an idea of the resistome of poultry carcasses and retail meat products and their potential to disseminate antimicrobial resistance humans, while most of the more recent studies have focused on the fecal microbiome. There are two studies that assessed the carcass or retail meat product using NGS for ARGs. Recently, De Cesare et al. (2022), performed a pilot study in the European Union where they characterized the caeca and corresponding carcasses of chickens raised conventionally, and chickens raised without antibiotics. They reported clear separation in the caeca samples between conventional and antibiotic free rearing for antimicrobial resistance, however they did not find any differences in the carcass resistome between the two production practices. They also noted that there was a greater antimicrobial resistance load on carcasses compared to the caeca and concluded that all post-harvest steps contributed to the resistance load on the final carcass that reaches consumers. In another study, Li et al. (2013) analyzed the resistome of retail chicken breasts, and reported that production practice (conventional vs antibiotic free) did effect the resistome of the chicken breast and that a majority of the 10 ARGs belonged to aminoglycosides and ß-lactams. They also determined the packaging

type of the chicken breasts did impact the resistome and found a low abundance of ARGs on chicken breasts compared to a later study by De Cesare et al. (2022). When De Cesare et al. (2022) compared their results back to Li et al. (2013), they attributed the differences they reported in ARGs to the regulatory differences between the United States and the European Union. While the earlier study by Li et al. (2013) ultimately concluded there was minimal risk of dissemination of ARGs to humans through the consumption of chicken breast regardless of production method, the more recent paper speculated that different parts of the harvest process all contribute to the ARGs on the chicken carcasses, and more research needs to be done at those different steps in the process.

*Beef*

The major pathogens of concerned in fresh beef are *Escherichia coli (specifically STECs), Salmonella* spp., *Staphylococcus aureus,* and *Listeria monocytogenes.* Of the 22% of foodborne illness cases that can be attributed to meat and poultry, only 13% of them can be attributed to beef products (Lianou et al., 2017). The beef industry has implemented many different prevention methods to decrease the risk of pathogens reaching consumers typically referred to as a "multiple hurdle approach". This can include both chemical and thermal treatments to mitigate the spread of pathogens.

In comparison to poultry, there were a greater number of studies that assessed the resistome of beef carcass and meat products. Similarly, to poultry the main question within those studies was if there were any differences in resistome based on production practice (conventionally vs. raised without antibiotics). Prior to the study by Noyes et al. (2016), there was very little research pertaining to ARGs in beef using shotgun metagenomics, where all prior work with ARGs in beef was performed using isolates. In that study they identified several different steps in the beef production system that had the potential to act as a vector for the transmission of ARGs to humans

both through the environment and the consumption of beef products. In addition to the pre-harvest environmental samples they took, (feces, soil, and water), they also took post-harvest swabs of the conveyor belt and rinsates of meat trimmings. They concluded that there was significantly few ARGs in the post-harvest samples and that the multiple hurdle intervention systems used during beef processing were effective in minimizing the likelihood of antimicrobial resistant infection through beef consumption. However, Noyes et al. (2016) noted there were high amounts of host DNA resulting in incomplete sequencing depths and suggested further investigation be done to negate the interference caused by the overwhelming amounts of host DNA to provide a more accurate resistome. Doster et al. (2020b) purchased packaged ground beef from local grocery stores as unlabeled or conventionally raised or labeled as raised without antibiotics, and they purchased these samples in a variety of different packaging styles (vacuum sealed, chub wrap, store grind and wrapped, and tray overwrap). The purpose of this study was to determine if there were any differences in the resistome of products raised conventionally or without antibiotics. However, unlike the previous study, they used a customized bait system termed "MEGaRICH" to target enrich for the resistome as developed in Noyes et al. (2017). This novel step allowed for the improvement of on target sequencing helping to sequence microbial DNA from samples that contained predominately host DNA and eliminating a lot of the noise caused by that host DNA. Doster et al. (2020b) reported there were no differences in the resistome of the ground beef samples in regard to the label on the product; however, they did report there being a difference in resistome of the ground beef products based on the type of packaging used. In a subsequent study by Weinroth et al. (2022), they also compared the two production practices of cull cows from conventionally raised beef and dairy cattle and organically raised dairy systems. They collected both colon and meat trimming rinsates from each different production style and metagenomic

DNA was obtained from the samples using target enriched sequencing to analyze the resistome. Weinroth et al. (2022) also reported there were no differences in the resistome of the meat trimmings between the production practices. In general, these results indicate production practice has minimal influence on the final meat products that are available to consumers, however, there is potential for facility and packaging type to play a key role in the dissemination of ARGs in beef products.

*Pork*

Just as with antibiotic usage in both poultry and beef cattle, antibiotics are utilized by the swine industry to treat disease and delivered in low doses through both water and feed to increase performance by increasing rate of gain and feed efficiency to meet the growing consumer demands (Cromwell, 2002). Similar to poultry, swine industry has shifted its focus to sustainability resulting in a shift away from antimicrobial usage (Zeineldin et al., 2019). Although, a great value is placed on sustainability, AMR transmission from swine to humans is still a concern in the industry. There have been numerous studies to evaluate the impact of antibiotic usage on ARGs throughout different levels of production in the swine industry.

In 2009, Kazimierczak et al. examined the effect of the discontinuation of antibiotic use on the ARGs in pigs. They conducted a metagenomic analyses of feces from pigs reared in an antibiotic free environment, to determine their resistome. Surprisingly, they reported there were ARGs sequenced in pigs that should represent a pristine environment free of antibiotics. They also brought attention this study being the first time that tet(40) had been found outside the human gut. In a later study Zwonitzer et al. (2016) sampled six different swine operations both antibiotic free and conventional farms where antibiotics are fed prophylactically. Conventional systems were significantly more resistant to amoxicillin, ampicillin, chlortetracycline, erythromycin,

kanamycin, neomycin, streptomycin, tetracycline, and tylosin, but also found resistant *Escherichia coli* in isolates from organic manure. Additionally, Lugsomya et al. (2018), compared the conventional and raised without antibiotics pigs to determine if there were any differences in ARGs. They found that *Escherichia coli* resistant to aminoglycosides and extended-spectrum ß-lactams (ESBL), were in greater abundance in conventional farms. On the other hand, Thakur and Gebreyes (2005) provided almost contrary results as they reported antimicrobial resistant *Campylobacter* was present in the manure of pigs raised both conventionally, and without antibiotics. In a study that assessed the subtherapeutic administration of tylosin and chlortetracycline on the ARGs in swine, pigs supplemented with tylosin had a significantly greater prevalence of tylosin resistant bacteria compared to non-supplemented pigs, and the ARGs tet(O), tet(Q), and erm(B) were detected in all pigs (Holman and Chénier, 2013). Due to the application of swine manure to crop land as a soil amendment it is important to fully understand how ARGs move through the environment.

While many studies have assessed the fecal resistome in pigs raised under various conditions, few studies have looked into the resistome of pork during or post-harvest. In the only study in pork assessing ARGs of pork in a retail store setting, Vikram et al. (2019) collected pork chops from animals raised without antibiotics and conventionally from food service suppliers and cultured *Escherichia coli*, tetracycline-resistant *Escherichia coli*, third-generation cephalosporin-resistant *Escherichia coli*, *Salmonella enterica*, tetracycline-resistant *Salmonella*, third-generation cephalosporin-resistant *Salmonella*, nalidixic acid–resistant *Salmonella*, *Enterococcus* spp., tetracycline-resistant *Enterococcus*, erythromycin-resistant *Enterococcus*, *Staphylococcus aureus*, and methicillin-resistant *Staphylococcus aureus*. They determined that production practice did not impact the detection of cultured bacteria. Additionally, quantitative PCR was used to assess the

aac(6′)-Ie-aph(2″)-Ia, aadA1, blaCMY-2, blaCTX-M, blaKPC-2, erm(B), mecA, tet(A), tet(B), and tet(M), and similarly, there were no differences between the swine production practices. The other two studies focused more on the resistome within the harvest facility. The first study by Liu et al. (2020), focused solely on resistance on salmonella in pork production systems in China. They isolated 105 Salmonella strains from pig carcasses and the environment of 4 separate harvest facilities. They reported that all isolates were multi-drug resistant with 95% being resistant to tetracyclines and 91% resistant to trimethoprim and sulfamethoxazole. A recent study by Cobo-Díaz et al. (2021) recorded the establishment of the microbial environment and resistome in the first 1.5 years of a newly built pork fabrication facility in Spain. They took samples of pork carcasses as well as environmental samples of the plant including belts, drains, and equipment in the processing area. They noted the resistome structure temporally evolved, and *Acinetobacter* and *Pseudomonas* were the most relevant carriers of ARGs and the most abundant ARGs associated with resistance were to betalactams (27.2%), tetracyclines (26.9%), aminoglycosides (25.3%), and quinolones (7.5%.). However, over time, there was significant increase an in the relative abundance of ARGs related to resistance to aminoglycosides, tetracyclines, and antimicrobials of the MLSP group (macrolides, lincosamides, streptogramins, and pleuromutilins) which are typically associated with swine. They also noted that mobile genetic elements were greater in greater abundance in drains and those mobile genetic elements increased over time. Ultimately, the researchers suggested that the change in resistome with dissemination of ARGs from the pork carcasses themselves determined they were a primary source of ARGs in the new facilities and played a key role in the built resistome in the plant.

## Final considerations

The use of next generation sequencing to evaluate the resistome different environments has offered great insight into the resistome of many different environments throughout agriculture. Numerous studies evaluated the fecal resistome of food production animals; however, few studies have evaluated the resistome of meat animal carcasses, and meat products. Of the studies that evaluated the resistome of meat animals, samples were taken either after fabrication, or in a retail setting. In order to fully understand all of the potential vectors of AMR during meat processing, there should be sampling during multiple points in the harvest process, as well as through packaging. As ground beef packaged in different packages have different resistome, it is clear there is limited knowledge of AMR dissemination at multiple points in the meat production process from harvest to the retail case.

# CHAPTER 3 - EVALUATION OF THE IMPACT OF AVIAN, BOVINE, AND PORCINE FECAL MICROBIOMES AND RESISTOMES ON SUBSEQUENT ENVIRONMENTS THROUGHOUT PRODUCTION


## Summary

The discovery of antibiotics for human and animal use is considered one of the greatest medical advancements. However, the widespread use of antibiotics has caused concerns about a potential increase of antimicrobial resistance genes (ARGs) within microbial communities. Microbes can acquire ARGs through horizontal gene transfer or mutations, resulting naturally and from environmental pressures. Many consumers consider antibiotic usage in animal agriculture to be a source for an increased abundance of antimicrobial resistant microorganisms in humans and the environment through meat consumption and manure application as a soil amendment. Therefore, the objective of this study was to evaluate the relationships between the fecal resistome of different food animal species (avian, bovine, and porcine), the resistomes of meat from those animals, and resistomes of soil where feces was used as an amendment.

Composite fecal samples ($n$ = 20 per species) were collected from each commercial production facility and meat rinsate samples were ($n$ = 20 per species) collected for each species at the time of harvest. After harvest, feces and litter were composted and applied as an amendment on agricultural land. Soil samples ($n$ = 20 per species) were collected separately for each species. Additionally, human waste solids were collected from wastewater treatment plants near each animal production operation ($n$ = 14 per species), and soil samples amended with human waste solids were collected ($n$ = 7 per species) from fields in close proximity to the broiler and bovine facilities. Metagenomic DNA obtained from samples were analyzed using target-enriched

sequencing to characterize the resistome and 16S rRNA gene sequencing to characterize the microbiome. Regardless of species, fecal samples had a greater ($P < 0.05$) richness and evenness of ARGs compared to both meat and soil samples. For beta diversity, all the sampling types clustered ($P < 0.05$) individually (feces, meat, and soil) within species. Furthermore, within species each environment was dominated by different classes of ARGs indicating they have different resistomes. When resistance groups medically important for human health were considered, human waste samples had a greater ($P < 0.05$) percentage (13%) of medically important resistance groups compared to all animal fecal samples (< 5%). The resistome of feces was richer and more diverse and clustered independently from both meat and soil indicating feces had a more unique resistome across the different species. This suggests that the fecal resistome is different from the meat and amended soil resistomes.

**Introduction**

The discovery of antibiotics for human and animal use is easily considered one of the greatest medical advancements in the past 100 years. However, antimicrobial resistant (AMR) bacteria are a worldwide public health concern, and according to the World Health Organization (WHO) and the United States Centers for Disease Control and Prevention (CDC) (World Health Organization, 2017; CDC, 2021). The widespread use of antibiotics in human and veterinary medicine has triggered concerns about a potential increase of antimicrobial resistance genes (ARGs) within microbial communities. Antibiotic usage in food animal production has been receiving increased criticism as a potential source for the spread of ARGs to humans directly through the consumption of meat and through the environment. Antibiotic resistance is a naturally occurring factor represented by the discovery of ARGs in ancient 30,000 year old permafrost. Bacteria can acquire ARGs through horizontal gene transfer or genetic mutations leading to the

spread of antimicrobial resistance (D'Costa et al., 2011). The objective of this study was to evaluate the relationships between the fecal microbiome and resistome of different food animal species (avian, bovine, and porcine), the microbiome and resistomes of meat from those animals, and the microbiome and resistomes of soil where feces was used as an amendment.

## Materials and Methods

### *Sample Collection*

Feedlot cattle, market hog, and broiler chicken production systems were sampled at a single location and time following the same group of animals. From each production system, animal feces ($n$ = 20 per species), meat ($n$ = 20 per species), and soil ($n$ = 20 per species) where animal feces was applied as a soil amendment after composting were collected. Additionally, treated human waste biosolids ($n$ = 14 per species) were collected from a nearby human wastewater treatment facility in close proximity to each animal system, and soil ($n$ = 7) where human waste biosolids were applied as a soil amendment in close proximity to the avian and bovine facility.

Fecal samples ($n$ = 80) were collected ≤ 48 h prior to the harvest and were composited to obtain 20 pooled samples, and meat trimmings were collected during carcass fabrication of the same cohort of animals. After animals were removed, the feces for each species was allowed to compost (1 to 4 months) and the composted manure from each production system was applied to nearby crop land as a soil amendment. The amended soil samples were then collected during crop harvest. Treated human waste biosolids were collected at the same point in time as animal feces from nearby wastewater treatment facilities. Additionally, a different subset of treated human wastewater was applied to grasslands and soil samples from the fields were collected after plant growth. All samples were frozen with dry ice and transported to Colorado State University and upon arrival were stored at -80°C until further processing.

*DNA Isolation and Sequencing*

The DNA was isolated directly from the feces, soil, and human waste biosolid samples while a surface rinsate was performed on meat samples. For all raw fecal samples from animal production facilities, fecal debris was removed using a pre-sedimentation step. For each 10 g sample, 30 ml of buffered peptone water was added to the conical tube and the tube was shaken until well mixed. Larger particles were allowed to settle out of suspension for approximately 5 to 10 minutes, and the supernatant was transferred to a clean conical tube. The conical tubes were then centrifuged at 4300 xg for 10 min, the supernatant was poured off and the resulting pellet was resuspended in 5 ml of 1x phosphate buffered saline (PBS). Samples were centrifuged and the supernatant was removed a final time. DNA was isolated from the pellet (sedimented or meat rinsate) or 10 g of non-sedimented sample using the PowerMax Soil DNA Isolation Kit (MoBio Laboratories, Inc.) according to manufacturer's instructions with some modifications. For meat trim samples, surface rinsates were performed by adding 180 mL of phosphate-buffered saline to the bag and each sample was hand massaged. Following massaging, all supernatant was removed from the bag and centrifuged (10,000 ×g for 10 min at 4°C). The resulting pellet was stored at -80°C until DNA extraction. DNA concentration was assessed using the Qubit dsDNA HS Assay Kit on a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA). DNA quality (260/280 ratio) was assessed using a NanoDrop ND-1000 (Thermo Fisher Scientific, Waltham, MA).

*16S rRNA Library preparation and sequencing*

For 16S rRNA amplicon sequencing, Aliquots of DNA were shipped to Novogene Corporation (Beijing, China) for library preparation and sequencing. The V4 region of the 16S subunit was amplified with the 515F/806R primer set [5'-GTGCCAGCMGCCGCGGTAA-3'] / [5'-GGACTACHVGGGTWTCTAAT-3']. Amplicon sequencing was performed using paired-end

chemistry (2 × 250 base pairs) on a HiSeq 2500 Sequencing System (Illumina, Inc., San Diego, CA) with a targeted depth of 100,000 reads per sample.

*Target Enriched AMR metagenomic Library Preparation and Sequencing*

For target enriched shotgun metagenomics, DNA libraries were prepared using the SureSelectXT Target Enrichment System for Illumina Paired-End Multiplexed Sequencing Library (Aligent Technologies, Santa Clara, CA) with a custom Antimicrobial Resistance gene target-capture library 'MEGaRICH' (Noyes et al., 2017). Libraries were sequenced (2 × 150 base pairs) on the HiSeq 2500 Sequencing System (Illumina, Inc. San Diego, CA). The sequencing depth on the meat samples was 4 samples per lane, soil samples were sequences at 15 samples per lane, and feces/biosolids were sequences at 20 samples per lane.

*Bioinformatics*

The 16S rRNA reads were received from Novogene and sequences were imported into and processed with QIIME2 version 2019.10 (Bolyen et al., 2019). Amplicon sequence variants (ASVs) were assigned using DADA2 (Callahan et al., 2017). Forward reads were trimmed at 25 base pairs and reverse reads were trimmed at 26 base pairs, while both were truncated at 230 base pairs. A Naïve Bayes Classifier was trained on the Greengenes database version 13_8 99 % (DeSantis et al., 2006) to assign taxonomy. Reads assigned to chloroplast or mitochondria were removed from the ASV table.

Antimicrobial Resistance Target enriched metagenomic sequence reads were processed using the AMR++ v2 pipeline and reads were aligned to the MEGARes v2 antimicrobial resistance database (Lakin et al., 2017; Doster et al., 2020a). Briefly, the AMR++ v2 pipeline utilized trimmomatic (Bolger et al., 2014) to trim and filter the reads for quality, and BWA was used to identify host DNA (*G. gallus*, *B. taurus*, S. *scrofa*, and *H. sapiens*) and align non-host reads to the

MEGARes antimicrobial resistance database. Gene sequences aligning to > 80% of the of the reference nucleotide sequence were included in further analysis. Reads aligning to gene accessions that require single nucleotide polymorphism confirmation were excluded from statistical analyses.

*Bioinformatics and statistical analyses*

Antimicrobial resistance genes identified as medically important when found in human pathogens in a previous study by (Noyes et al., 2016a) were identified. These represented 19 different antimicrobial resistance gene groups.

Data (16S rRNA ASV and AMR-TE ARG count tables) were imported separately in to R version 4.0.4 (R Core Team, 2018) using the phyloseq (McMurdie and Holmes, 2013) 'import_biom' function and metadata was merged with each count table to form a phyloseq object. Alpha diversity metrics (richness, and Shannon's) were calculated with phyloseq and Pielou's evenness index (Pielou, 1966) was calculated using the microbiome package (Lahti and Shetty, 2017). Count tables (ASV and ARG) were normalized using cumulative sum scaling (Paulson et al., 2013). Beta-diversity for ASVs was analyzed using generalized UniFrac distances (Chen et al., 2012), while beta-diversity for ARGs was calculated using Bray-Curtis dissimilarity distances (Ricotta and Podani, 2017). Non-metric multidimensional was performed on the distances and plotted. Significant differences in community structure were calculated using a permutational multivariate analysis of variance (PERMANOVA) with the vegan (Oksanen et al., 2019) and pairwiseAdonis (Arbizu, 2019) packages. Hierarchal clustering was performed using Ward's agglomeration clustering method (Murtagh and Legendre, 2014) using the 'hclust' function for both generalized UniFrac and Bray-Curtis distances. The relative abundances of both 16S and AMR-TE were calculated using phyloseq. Pairwise Wilcoxon rank-sum tests were performed with a Benjamini-Hochberg (Benjamini and Hochberg, 1995) correction for multiple post hoc

comparisons, and differences in beta-diversity were analyzed using pairwise PERMANOVA with a Benjamini-Hochberg correction for multiple comparisons and 9,999 permutations. For all analyses, critical alpha was set at 0.05.

## Results

*Resistome and microbiome composition*

A total of 236 samples were target-enriched and sequenced resulting in an average of 41,251,699 paired-end clusters per sample (ranging from 1.5 million – 287.6 million). There were 3,277 different gene classifications representing 50 drug classes, and 156 resistance mechanisms. Across all sample types (feces, soil, meat, and human-waste biosolids) and species (avian, bovine, porcine, and human) the most abundant resistance classes were tetracyclines (21.3%), multi-metal resistance (16.1%), drug and biocide resistance (16.0%), macrolide-lincosamide-streptogramin (5.9%), betalactams (5.7%), aminoglycosides (5.0%), mercury resistance (5.0%), copper resistance (4.1%), and sulfonamides (3.8%). Additionally, the 16S rRNA gene was sequenced for 223 samples. There was an average of 128,619 reads per sample which resulted in a total 57,229 different amplicon sequence variances. Proteobacteria was most common phylum in both soil (44.1%) and meat (55.1%) and firmicutes were the most common phyla in fecal samples (42.3%) regardless of the host species.

*Resistome and Microbiome*

Regardless of production operation (avian, bovine, and porcine) the resistome of fecal samples was richer and more diverse compared to both meat and soil samples where composted feces was used as a soil amendment as evidenced by a greater richness, Pielou's evenness, and Shannon's alpha diversity metric ($P < 0.05$; Figures 3.1 – 3.3). This pattern was also present in human samples as waste biosolids collected from waste treatment facilities in close proximity to

each production facility displayed a greater richness, Pielou's evenness, and Shannon's alpha diversity index compared to soil samples where human waste were used as a soil amendment ($P < 0.05$; Figure 3.4). While the resistome of fecal and human waste biosolid samples were richer and more diverse, this was not the case with the 16S microbial structure. For all production operations, amended soil samples had a greater richness, Pielou's evenness, and Shannon' Alpha Diversity Index ($P < 0.05$; Figures 3.5 – 3.7). This was also evident in the human samples as soil samples amended with human waste biosolids had a greater richness, Pielou's evenness, and Shannon's Alpha Diversity Index ($P < 0.05$; Figure 3.8).

There were also significant differences in both resistome and microbial community structure. The resistome beta diversity of each sampled environment (feces, meat, and soil) within each animal production system were different based on Bray-Curtis dissimilarity values indicating the composition of ARGs across each environment was different (PERMANOVA $P < 0.05$; Figure 3.9.A, B, C). Additionally, the beta diversity of human derived resistome samples showed significant differences by environment (waste biosolids and amended soil; PERMANOVA $P < 0.05$; Figure 3.9.D) and location of the sampling (in close proximity to avian, bovine, and porcine production facilities; PERMANOVA $P < 0.05$). The microbial community showed similar differences between environments (Figure 3.10). The beta diversity of each sampled environment (feces, meat, and soil) within each animal production system were not similar based on Generalized UniFrac distances indicating the microbial community of each environment was unique (PERMANOVA $P < 0.05$; Figure 3.10.A, B, C).

Similarly, this was also demonstrated in the microbial communities of the human derived biosolid and soil samples as there were significant differences in both environment and location (PERMANOVA $P < 0.05$; Figure 3.10.D). These resistome and microbial structure differences

were also evident when hierarchal clustering was performed. For both ARGs and 16S there was distinct clustering of samples within each environment. Interestingly, within the resistome and microbiome, these clusters followed the same pattern observed in the alpha diversity metrics. Hierarchal clustering of the resistome, showed across all host species, fecal samples formed an independent clade apart from both soil and meat samples (Figure 3.11 - 14.A). Conversely, in the microbiome hierarchal clusters across species, soil formed clades independently of both meat and fecal samples. (Figure 3.15 - 18.A). Additionally, for both the resistome and microbiome hierarchal clustering showed there were unique clades formed for human waste biosolids and amended soil, and within each environment there was further clustering among location of the water treatment facility (avian, bovine, and porcine).

*Avian resistome and microbiome*

The most prominent class of ARGs found in avian litter samples was tetracyclines (31.5%), and this was significantly greater than the abundance of tetracycline ARGs found in meat (13.4%) and soil (4.6%; Figure 3.19A). The predominant tetracycline groups associated with the litter resistome were TETW (8.7%), TETM (7.9%), TETO (5.9%), and TETQ (1.8%), which were all significantly lower in meat and soil samples ($P < 1.0\%$; $P < 0.05$; Figure 3.20.A). In meat samples, the most common tetracycline group was TET39 (7.7%) which was significantly greater than both feces and soil (< 0.5%; $P < 0.05$). The groups TETW, TETM, TETO, and TETQ all confer resistance to ribosomal protection proteins, while TET 39 which was most common in meat encoded for MFS efflux pumps. Determinants conveying multi-metal resistance were the most prominent class in avian meat trim samples (29.0%; $P < 0.05$), significantly greater than both litter (10.9%) and soil (9.1%) which were similar ($P > 0.05$). Additionally, Drug and biocide resistance was the most prominent resistance class in soil samples amended with composted avian litter

(37.3%), compared to litter (11.3%) and feces (15.4%). Firmicutes were the most dominant phyla in the avian litter samples (65.9%), while proteobacteria was the most common in both soil (46.8%) and meat (78.1%; Figure 3.22.A).

*Bovine resistome and microbiome*

The bovine fecal resistome showed the greatest abundance of determinants for tetracycline resistance with 48.9% compared to both meat (3.6%) and soil (5.5%) samples (Figure 3.19.B). Bovine fecal samples had a greater abundance of TETW (15.4%), TETO (13.1%), TETM (8.0%), and TETQ (4.0%), when compared to both meat and soil samples ($P < 0.05$%, Figure 3.20.A). Bovine meat trimmings and soil samples showed less than 1% of tetracycline groups TETW, TETM, and TETO, and less than 2% of TETQ. The bovine meat resistome was dominated by genes conferring for multi-metal resistance (70.2%), which was significantly greater than fecal (6.7%) and soil (6.6%) samples ($P < 0.05$). Additionally, the resistome of soil samples amended with composted bovine feces had a greater abundance of drug and biocide resistant groups (33.2%) compared to feces (9.5%) and meat trimmings (1.7%; $P < 0.05$, Figure 3.7). The microbiome of the bovine fecal samples consisted mostly of firmicutes (64.0%), and bacteroidetes (17.1%, Figure 3.22.B). The meat trim samples were dominated by proteobacteria (55.9%) and firmicutes (24.5%), while soil consisted of proteobacteria (42.9%), actinobacteria (12.2%), acidobacteria (12.2%), and gemmatimonadetes (10.0%).

*Porcine resistome and microbiome*

The most prominent resistance class in porcine fecal samples were tetracyclines (41.2%) of which, most prominent resistance groups were TETW (13.9%), TETO (11.4%), TETQ (3.5%), and TETM (2.8%, Figures 3.19.C and 3.20.A). The abundance of tetracycline resistant genes feces (41.2%) was similar to the abundance of tetracycline resistant genes in porcine meat samples

(39.2%, P = 0.99); however, the meat trimming contained a greater percentage TETQ (24.6%) and TET39 (10.1%). Porcine meat trim samples also contained the greatest abundance of genes conferring multi-metal resistance (36.2%) compared to fecal (8.4%) and soil (6.6%) samples ($P < 0.05$). Soil samples amended with composted porcine feces revealed the greatest abundance of genes conferring drug and biocide resistance (31.1%) compared to feces (10.3%) and meat (2.6%; $P < 0.05$). The porcine fecal microbiome was 58.2% firmicutes, 19.8% Bacteroidetes (Figure 3.22.C). The microbiome of the meat trim samples were dominated by proteobacteria (35.5%) and firmicutes (33.3%), and the soil microbiome consisted of proteobacteria (47.2%), acidobacteria (13.2%), actinobacteria (13.2%), and Bacteroidetes (7.3%).

*Human waste biosolids resistome and microbiome*

The human waste biosolid samples had a greater abundance of tetracycline resistance ARGs (23.5%) compared to soil (3.6%; $P < 0.05$; Figure 3.19.D). the most common genes in human biosolid samples were TETW (5.8%), TETO (4.8%), TETQ (2.6%) and TETC (2.6%), and these were all in greater percentages than in soil samples where they were present in less than 1% ($P < 0.05$, Figure 3.20.A).Additionally, sulfonamides (14.8%), betalactamases (14.6%), MLS (12.3%), and aminoglycosides (10.4%) were abundant in the human biosolid samples. Conversely, genes coding for drug and biocide resistance (27.6%) were the most abundant in soil samples amended with composted human biosolids ($P < 0.05$). The human waste biosolids microbiome, primarily consisted of proteobacteria (32.3%), bacteroidetes (21.9%), and firmicutes (13.3%). Additionally, the soil amended with human waste, was dominated by proteobacteria (37.4%) and actinobacteria (17.2%), and firmicutes (11.4%; Figure 3.22.D).

*Medically important antimicrobial resistance genes*

There were 19 genes identified *a priori* as being medically important to human health when expressed in human pathogens. Of these 19 genes, across all production systems, and human derived samples, 12 of them were identified across all species and sample types. There were 11 medically important ARGs found in avian samples, 9 medically important ARGs found in porcine samples and 10 medically important ARGs found in bovine and human derived samples. There were no ARGs found in: *bla*(IMI), *bla*(MEC),*bla*(NDM), *bla*(SME), *mcr* (colistin phosphoethanolamine transferase) , *vat* (streptogramin A O-acetyltransferase), and *vgb* (streptogramin B ester bond cleavage). Alignments to *bla*(KPC) were only found in one of the human biosolid samples. Overall, genes conferring resistance to beta lactamases were the most abundant class of medically important determinants identified across all sample types.

Overall, there was a significantly greater percentage of ARGs considered medically important to human health in human waste biosolid samples (11.96%) compared to fecal samples from meat animal production systems (0.73% - 2.19 %; $P < 0.05$; Figure 3.23). The soil samples amended with manure and human waste biosolids were dominated by *sme* (avian = 89.2%, bovine = 96.1%, porcine = 90.5%, and human = 84.3%). Additionally, when comparing the medically important ARGs of production animal fecal samples to the human waste biosolid samples, they were found to be significantly different from one each other with the biosolid samples clustering independently from the animal fecal samples ($P < 0.05$; Figure 3.26).

**Discussion**

This study provides a unique opportunity to assess the resistome and microbiome of three different potential vectors of antimicrobial resistance across a boiler chicken, beef, and pork production system through the sampling of feces, soil amended with composted manure, and meat

trimmings. Additionally, this study provided an overview of the potential resistance conveyed in human fecal samples via the sampling of human waste biosolids, and the soil of land amended with those human waste biosolids. To date no other study has provided such a wide range of insight into the ARGs throughout multiple food animal production systems. The target enriched metagenomic data indicated that within each species, each production environment was different furthering the idea that AMD use in livestock production poses a lesser risk of potential human exposure to antimicrobial resistant bacterial populations via meat consumption or through amendment of land with manure from productions systems where AMDs are used. This is similar to a study by Hu et al. (2016), where they reported that animal feces, human waste biosolids, and soil microbiomes clustered independently on an ordination plot and identified these individual microbiomes as possibly preventing the dissemination of ARG between each other.

Based on the results of previous studies, it was expected for the resistome and microbiome of fecal samples to be significantly different from the meat trimming and amended soil for each species which is what the current study shows. For all animal species and human waste biosolids, tetracyclines were the most prominent resistance class across all species consisting primarily of the ARGs tet(O), tet(M), tet(Q), and tet(W). The high abundance of tetracycline resistance is in line with previous work in in avian, bovine, and porcine species (Kazimierczak et al., 2009; Holman and Chénier, 2013; Schmidt et al., 2020; Doster et al., 2021; De Cesare et al., 2022; Weinroth et al., 2022). In this study, there was also a high prevalence of drug and biocide, aminoglycoside, and multi-metal resistance and MLS in all animal feces. In the human waste biosolids, tetracyclines were also an abundant resistance class, along with aminoglycosides, and MLS, as well as betalactams and sulfonamides which were not as prevalent in the animal feces. The gene *bla*(OXA) was the most abundant betalactam resistance gene in human waste biosolids.

These results show similar patterns to other studies that have looked at the microbiome and resistome of individual production systems where each environment was unique. Regardless within each species, the meat trimmings had a unique resistome and microbiome separate from both the fecal samples and amended soil samples. In the current study, multi-metal resistance was reported to be the most abundant class for both avian and bovine meat trimmings, while porcine trimmings had a greater abundance of tetracycline and multi-metal resistance classes. The results for avian and bovine are different from previous studies. A previous study using similar target enriched sequencing methods to investigate meat trimmings in cull cows found tetracyclines to be the most prominent class, and another study of the resistome of ground beef in retail stores also found tetracyclines to be the most prominent resistance class (Doster et al., 2020b; Weinroth et al., 2022). In a previous study to observe the resistome of chicken breast samples Li et al. (2020) found the majority of the resistome on the retail chicken breast samples belonged to the resistance class aminoglycosides and betalactams. In contrast, while aminoglycosides and betalactams were both present in the chicken samples of the currently study, they were not the most noteworthy classes. Additionally, a pilot study by De Cesare et al. (2022) where they tested resistome chicken carcasses, also reported aminoglycosides and betalactams as the most prominent classes on the broiler carcasses. In the current study, tetracyclines and multi-metal resistance were the most abundant classes in the swine meat trimmings. Several other studies in pork have also reported tetracyclines as a predominant class (Vikram et al., 2019; Liu et al., 2020; Cobo-Díaz et al., 2021). While there are no other references to multi metal resistance in meat trimmings, it has been mentioned in different resistome studies of livestock as a result of metal supplementation in animal feed (Guo et al., 2021; Tunsagool et al., 2021). Another noteworthy resistance class prevalent in the meat samples from all three production animals was mercury resistance. H. Yang et al. (2020),

where they isolated antibiotic and metal resistant *Escherichia coli* in pork harvest facilities in the United Kingdom, and reported finding resistance to mercury, silver, and copper with 18% of strains being resistant to mercury.

While some of the previous literature has suggested the presence of ARGs in manure correspond directly with the usage of antibiotics during animal production (Topp et al., 2018). Our results in the current study suggested manure and biosolids application on soil as an amendment had very little impact on the microbiome and resistome of the soil. Regardless of the manure application, the soil microbiome was dominated by proteobacteria, actinobacteria, and acidobacteria. Additionally, soil resistome was consistent regardless of which manure application was applied. Drug and biocide resistance was the most abundant resistance class across all amended soil samples. These results are consistent with results of Chen et al. (2019) where they compared the antimicrobial resistance gene profiles amongst soils according to application, and they reported the environmental resistome risk scores of all amended soils including manure from animal treated with antibiotics were equivalent to that of the unamended soils. However, Macedo et al. (2020) tested soil with different textures to determine its ability to recover its beginning levels of ARGs after manure application, and determined that manure applications increased *erm(*B) and *tet*(W) in the soil, and the type of soil impacted its ability to recover at a certain rate; however, final recovery times were predicted to occur between 29 – 42 days.

Due to their importance to human health, 19 ARG groups were identified as being medically important to human medicine. When these resistance genes are present in pathogenic bacteria, they can cause infections that are difficult to treat with current antibiotics posing a serious threat to public health. The medically important ARG groups are based off the ARGs described in Noyes et al. (2016b). Of the 19 medically important ARG groups, 12 of them were identified in at

least one sample of the current study and most notably, human waste biosolids possessed a significantly greater abundance of medically important ARGs (12%) compared to the feces collected from each animal production operation (0.7% to 2.2 %). Additionally, when the fecal resistome of the food animals was compared to the human biosolid resistome on an ordination plot, the animal feces clustered independently of the human was biosolids indicating the biosolids had a different resistome of medically important ARGs compared to the feces all three species of meat animal

**Conclusions**

Currently, there are no other metagenomic studies that evaluate the impact of the fecal microbiome and resistome on both meat and manure amended crop lands to understand the effect ARGs in three different meat production animal operations could have on potential vectors for the transmission of AMR to humans. This study provides substantial evidence to indicate that the conclusions in previous studies suggesting there is minimal dissemination of ARGs to subsequent meat and soil environments of conventionally produced meat animals. Furthermore, the increased abundance of medically important ARGs in human waste biosolids could indicate contact with human waste is of greater risk for the dissemination of ARGs to subsequent environments, and further investigation should be done to fully understand the impact of human use of antibiotic on environmental resistomes.

## Tables

**Table 3.1** Antimicrobial resistance gene sequencing quality

| Sequencing Quality for Antimicrobial Resistance Genes | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | **Environment** | **Paired Reads** | **Raw Reads** | **Forward Reads** | **Reverse Reads** | **Reads Removed** | **Reads Passing Quality Control** | **Mapped to Host** | **Host (%)** | **Non-Host** | **Non-Host (%)** | **MEGARes[1]** | **MEGARES[1] (%)** | **DEDUP AMR[2]** | **AMR[3] (%)** |
| **Avian** | **Feces** | 33733650 | 67467300 | 795163 | 121193 | 50180 | 65535009 | 14531859 | 15.6542 | 51003150 | 84.3458 | 19867359 | 36.2978 | 1146573 | 2.2387 |
| | **Meat** | 71196340 | 142392680 | 2355455 | 253809 | 111591 | 137417221 | 127027197 | 89.4529 | 10390024 | 10.5471 | 836806 | 11.2787 | 70741 | 0.0696 |
| | **Soil** | 40155353 | 80310706 | 998022 | 95953 | 53200 | 78029622 | 7039039 | 9.5359 | 70990583 | 90.4641 | 6343050 | 6.3541 | 222586 | 0.2762 |
| **Bovine** | **Feces** | 33388989 | 66777979 | 829401 | 104143 | 52272 | 64807519 | 25845617 | 37.8685 | 38961903 | 62.1315 | 20055494 | 44.5736 | 752431 | 1.4821 |
| | **Meat** | 65291409 | 130582818 | 2401390 | 186893 | 105659 | 125774194 | 117496128 | 93.8477 | 8278066 | 6.1523 | 329143 | 13.7837 | 21993 | 0.0298 |
| | **Soil** | 25617182 | 51234364 | 620698 | 62156 | 32657 | 49820295 | 5810863 | 12.9595 | 44009433 | 87.0405 | 1256399 | 3.5393 | 79420 | 0.2227 |
| **Porcine** | **Feces** | 45477528 | 90955057 | 1357625 | 137322 | 149803 | 87673001 | 27674444 | 30.3360 | 59998557 | 69.6640 | 16874779 | 37.6900 | 853078 | 1.6947 |
| | **Meat** | 83601894 | 167203788 | 3920695 | 266770 | 142097 | 159414686 | 158576670 | 99.4529 | 838015 | 0.5471 | 35994 | 3.4311 | 8499 | 0.0059 |
| | **Soil** | 27703732 | 55407464 | 901707 | 66397 | 45352 | 53396050 | 4509614 | 8.8853 | 48886436 | 91.1147 | 1467664 | 3.3320 | 91020 | 0.1969 |
| **Human** | **Biosolids** | 21916439 | 43832878 | 648312 | 58866 | 45878 | 42329115 | 4253728 | 9.2253 | 38075387 | 90.7747 | 4060865 | 11.6990 | 351060 | 0.9545 |
| | **Soil** | 23106535 | 46213070 | 478257 | 42744 | 18748 | 45145741 | 4607262 | 11.3548 | 40538479 | 88.6452 | 3459126 | 8.0651 | 167802 | 0.4617 |
| **Total Average** | | 41251699 | 82503399 | 1338796 | 122376 | 72001 | 79602748 | 42041256 | 52.8138 | 37561492 | 64.1936 | 6695103 | 16.2740 | 350640 | 0.7302 |
| **Total Minimum** | | 1578905 | 3157810 | 22330 | 3554 | 697 | 3072900 | 12120 | 0.0473 | 261820 | 0.1795 | 72 | 0.0091 | 55 | 0.0000 |
| **Total Maximum** | | 287603137 | 575206274 | 10343821 | 782339 | 1644167 | 549788951 | 533845613 | 99.8205 | 403215315 | 99.9527 | 97022902 | 79.4384 | 1614465 | 4.1585 |

[1]MEGARes – The number of reads aligned to the MEGARes database.
[2]MEGARes (%) – The percentage of reads aligned to the MEGARes database.
[3]DEDUP AMR – Removal of duplicated antimicrobial resistant isolates.
[4]AMR (%) – The total percentage of antimicrobial resistant reads aligned and identified.

**Table 3.2** The 19 ARG[1] groups identified in avian samples *a priori* that are medically important when found in human pathogens. The percent of important ARGs and total ARGs reports the mean values within each group.

| | | | Avian | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Feces | | | Meat | | | Soil | | |
| Gene Group | Resistance Class | Resistance Mechanism | Percent Important ARGs | Percent Total ARGs | Samples with hits | Percent Important ARGs | Percent Total ARGs | Samples with hits | Percent Important ARGs | Percent Total ARGs | Samples with hits |
| CFR | Multi-drug resistance | 23S rRNA methyltransferases | 0.110 | 0.002 | 1/20 | 0.000 | 0.000 | 0/20 | 0.000 | 0.000 | 0/20 |
| CMY | β -lactamases | Class C β-lactamases | 0.685 | 0.014 | 5/20 | 0.000 | 0.000 | 0/20 | 0.602 | 0.032 | 8/20 |
| CPHA | β -lactamases | Class B β-lactamasesf | 0.000 | 0.000 | 0/20 | 0.073 | 0.004 | 2/20 | 0.000 | 0.000 | 0/20 |
| CTX | β -lactamases | Class A β-lactamases | 17.653 | 0.3541 | 20/20 | 39.698 | 1.957 | 20/20 | 5.821 | 0.312 | 17/20 |
| GES | β -lactamases | Class A β-lactamases | 0.423 | 0.008 | 3/20 | 0.000 | 0.000 | 0/20 | 0.057 | 0.003 | 1/20 |
| IMI | β -lactamases | Class A β-lactamases | - | - | - | - | - | - | - | - | - |
| IMP | β -lactamases | Class B β-lactamases | 0.000 | 0.000 | 0/20 | 0.000 | 0.000 | 0/20 | 0.584 | 0.031 | 7/20 |
| KPC | β -lactamases | Class A β-lactamases | - | - | - | - | - | - | - | - | - |
| MCR | Lipopeptide | Colistin phosphoethanolamine transferase | - | - | - | - | - | - | - | - | - |
| MEC | β -lactamases | Penicillin binding protein | - | - | - | - | - | - | - | - | - |
| NDM | β -lactamases | Class B β-lactamases | - | - | - | - | - | - | - | - | - |
| OXA | β -lactamases | Class D β-lactamases | 1.275 | 0.026 | 13/20 | 53.766 | 2.651 | 20/20 | 3.669 | 0.197 | 12/20 |
| SHV | β -lactamases | Class A β-lactamases | 0.000 | 0.000 | 0/20 | 0.000 | 0.000 | 0/20 | 0.004 | 0.000 | 1/20 |
| *bla*(SME) | β -lactamases | Class A β-lactamases | - | - | - | - | - | - | - | - | - |
| SME | Multi-drug resistance | Multi-drug RND efflux pumps | 0.000 | 0.000 | 0/20 | 5.611 | 0.277 | 8/20 | 89.200 | 4.782 | 19/20 |
| TEM | β -lactamases | Class A β-lactamases | 79.104 | 1.587 | 20/20 | 0.784 | 0.042 | 9/20 | 0.003 | 0.000 | 1/20 |
| VAT | MLS | Streptogramin A O-acetyltransferase | - | - | - | - | - | - | - | - | - |
| VGA | Multi-drug resistance | Multi-drug ABC efflux pumps | 0.749 | 0.015 | 7/20 | 0.000 | 0.000 | 0/20 | 0.073 | 0.004 | 1/20 |
| VGB | MLS | Streptogramin B ester bond cleavage | - | - | - | - | - | - | - | - | - |

**Table 3.3** The19 ARG[1] groups identified in bovine samples *a priori* that are medically important when found in human pathogens. The percent of important ARGs and total ARGs reports the mean values within each group.

| | | | Bovine | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Feces | | | Meat | | | Soil | | |
| Gene Group | Resistance Class | Resistance Mechanism | Percent Important ARGs | Percent Total ARGs | Samples with hits | Percent Important ARGs | Percent Total ARGs | Samples with hits | Percent Important ARGs | Percent Total ARGs | Samples with hits |
| CFR | Multi-drug resistance | 23S rRNA methyltransferases | 4.246 | 0.031 | 14/21 | 0.000 | 0.000 | 0/19 | 0.000 | 0.000 | 0/20 |
| CMY | β -lactamases | Class C β-lactamases | 0.000 | 0.000 | 0/21 | 0.000 | 0.000 | 0/19 | 0.181 | 0.005 | 1/20 |
| CPHA | β -lactamases | Class B β-lactamasesf | - | - | - | - | - | - | - | - | - |
| CTX | β -lactamases | Class A β-lactamases | 57.227 | 0.416 | 21/21 | 28.103 | 1.238 | 15/19 | 2.090 | 0.062 | 3/20 |
| GES | β -lactamases | Class A β-lactamases | 1.770 | 0.013 | 4/21 | 0.000 | 0.000 | 0/19 | 0.000 | 0.000 | 0/20 |
| IMI | β -lactamases | Class A β-lactamases | - | - | - | - | - | - | - | - | - |
| IMP | β -lactamases | Class B β-lactamases | 0.000 | 0.000 | 0/21 | 0.000 | 0.000 | 0/19 | 0.257 | 0.007 | 2/20 |
| KPC | β -lactamases | Class A β-lactamases | - | - | - | - | - | - | - | - | - |
| MCR | Lipopeptide | Colistin phosphoethanolamine transferase | - | - | - | - | - | - | - | - | - |
| MEC | β -lactamases | Penicillin binding protein | - | - | - | - | - | - | - | - | - |
| NDM | β -lactamases | Class B β-lactamases | - | - | - | - | - | - | - | - | - |
| OXA | β -lactamases | Class D β-lactamases | 3.406 | 0.025 | 19/21 | 54.630 | 66.451 | 19/19 | 0.185 | 0.005 | 1/20 |
| SHV | β -lactamases | Class A β-lactamases | 0.000 | 0.000 | 0/21 | 0.861 | 1.047 | 1.19 | 0.000 | 0.000 | 0/20 |
| *bla*(SME) | β -lactamases | Class A β-lactamases | - | - | - | - | - | - | - | - | - |
| SME | Multi-drug resistance | Multi-drug RND efflux pumps | 0.000 | 0.000 | 0/21 | 0.000 | 0.000 | 0/19 | 96.146 | 2.849 | 13/20 |
| TEM | β -lactamases | Class A β-lactamases | 29.136 | 0.212 | 9/21 | 4.399 | 3.616 | 7/19 | 1.141 | 0.034 | 4/20 |
| VAT | MLS | Streptogramin A O-acetyltransferase | - | - | - | - | - | - | - | - | - |
| VGA | Multi-drug resistance | Multi-drug ABC efflux pumps | 4.216 | 0.031 | 11/21 | 0.000 | 0.000 | 0/19 | 0.000 | 0.000 | 0/20 |
| VGB | MLS | Streptogramin B ester bond cleavage | - | - | - | - | - | - | - | - | - |

**Table 3.4** The 19 ARG[1] groups identified in porcine samples *a priori* that are medically important when found in human pathogens. The percent of important ARGs and total ARGs reports the mean values within each group.

| | | | Porcine | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Feces | | | Meat | | | Soil | | |
| Gene Group | Resistance Class | Resistance Mechanism | Percent Important ARGs | Percent Total ARGs | Samples with hits | Percent Important ARGs | Percent Total ARGs | Samples with hits | Percent Important ARGs | Percent Total ARGs | Samples with hits |
| CFR | Multi-drug resistance | 23S rRNA methyltransferases | 2.437 | 0.0534 | 18/20 | 0.000 | 0.000 | 0/20 | 0.000 | 0.000 | 0/20 |
| CMY | β -lactamases | Class C β-lactamases | 0.103 | 0.002 | 1/20 | 0.000 | 0.000 | 0/20 | 0.000 | 0.000 | 0/20 |
| CPHA | β -lactamases | Class B β-lactamasesf | - | - | - | - | - | - | - | - | - |
| CTX | β -lactamases | Class A β-lactamases | 15.892 | 0.348 | 20/20 | 12.012 | 0.250 | 3/20 | 2.836 | 0.058 | 5/20 |
| GES | β -lactamases | Class A β-lactamases | 1.320 | 0.029 | 3/20 | 0.000 | 0.000 | 0/20 | 0.000 | 0.000 | 0/20 |
| IMI | β -lactamases | Class A β-lactamases | - | - | - | - | - | - | - | - | - |
| IMP | β -lactamases | Class B β-lactamases | 0.186 | 0.004 | 1/20 | 0.000 | 0.000 | 0/20 | 3.896 | 0.080 | 9/20 |
| KPC | β -lactamases | Class A β-lactamases | - | - | - | - | - | - | - | - | - |
| MCR | Lipopeptide | Colistin phosphoethanolamine transferase | - | - | - | - | - | - | - | - | - |
| MEC | β -lactamases | Penicillin binding protein | - | - | - | - | - | - | - | - | - |
| NDM | β -lactamases | Class B β-lactamases | - | - | - | - | - | - | - | - | - |
| OXA | β -lactamases | Class D β-lactamases | 30.032 | 0.658 | 18/20 | 54.063 | 1.127 | 10/20 | 2.729 | 0.056 | 12/20 |
| SHV | β -lactamases | Class A β-lactamases | - | - | - | - | - | - | - | - | - |
| *bla*(SME) | β -lactamases | Class A β-lactamases | - | - | - | - | - | - | - | - | - |
| SME | Multi-drug resistance | Multi-drug RND efflux pumps | 1.671 | 0.037 | 1/20 | 0.000 | 0.000 | 0/20 | 90.539 | 1.863 | 17/20 |
| TEM | β -lactamases | Class A β-lactamases | 45.176 | 0.990 | 18/20 | 33.925 | 0.707 | 2/20 | 0.000 | 0.000 | 0/20 |
| VAT | MLS | Streptogramin A O-acetyltransferase | - | - | - | - | - | - | - | - | - |
| VGA | Multi-drug resistance | Multi-drug ABC efflux pumps | 3.182 | 0.0697 | 17/20 | 0.000 | 0.000 | 0/20 | 0.000 | 0.000 | 0/20 |
| VGB | MLS | Streptogramin B ester bond cleavage | - | - | - | - | - | - | - | - | - |

**Table 3.5** The 19 ARG[1] groups identified in human samples *a priori* that are medically important when found in human pathogens. The percent of important ARGs and total ARGs reports the mean values within each group.

| | | | Human | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Waste Biosolids | | | Soil | | |
| Gene Group | Resistance Class | Resistance Mechanism | Percent Important ARGs | Percent Total ARGs | Samples with hits | Percent Important ARGs | Percent Total ARGs | Samples with hits |
| CFR | Multi-drug resistance | 23S rRNA methyltransferases | 0.014 | 0.002 | 1/42 | 0.000 | 0.000 | 0/14 |
| CMY | β -lactamases | Class C β-lactamases | 0.136 | 0.016 | 30/42 | 0.158 | 0.006 | 1/14 |
| CPHA | β -lactamases | Class B β-lactamasesf | - | - | - | - | - | - |
| CTX | β -lactamases | Class A β-lactamases | 2.398 | 0.287 | 40/42 | 0.689 | 0.026 | 6/14 |
| GES | β -lactamases | Class A β-lactamases | 15.701 | 1.878 | 35/42 | 0.000 | 0.000 | 0/14 |
| IMI | β -lactamases | Class A β-lactamases | - | - | - | - | - | - |
| IMP | β -lactamases | Class B β-lactamases | 0.412 | 0.049 | 11/42 | 0.098 | 0.004 | 2/14 |
| KPC | β -lactamases | Class A β-lactamases | 0.001 | 0.0001 | 1/42 | 0.000 | 0.000 | 0/14 |
| MCR | Lipopeptide | Colistin phosphoethanolamine transferase | - | - | - | - | - | - |
| MEC | β -lactamases | Penicillin binding protein | - | - | - | - | - | - |
| NDM | β -lactamases | Class B β-lactamases | - | - | - | - | - | - |
| OXA | β -lactamases | Class D β-lactamases | 77.648 | 9.286 | 42/42 | 14.621 | 0.546 | 13/14 |
| SHV | β -lactamases | Class A β-lactamases | - | - | - | - | - | - |
| *bla*(SME) | β -lactamases | Class A β-lactamases | - | - | - | - | - | - |
| SME | Multi-drug resistance | Multi-drug RND efflux pumps | 3.432 | 0.410 | 30/42 | 84.348 | 3.151 | 13/14 |
| TEM | β -lactamases | Class A β-lactamases | 0.243 | 0.029 | 12/42 | 0.087 | 0.003 | 3/14 |
| VAT | MLS | Streptogramin A O-acetyltransferase | - | - | - | - | - | - |
| VGA | Multi-drug resistance | Multi-drug ABC efflux pumps | 0.015 | 0.002 | 1/42 | 0.000 | 0.000 | 0/14 |
| VGB | MLS | Streptogramin B ester bond cleavage | - | - | - | - | - | - |

## Figures

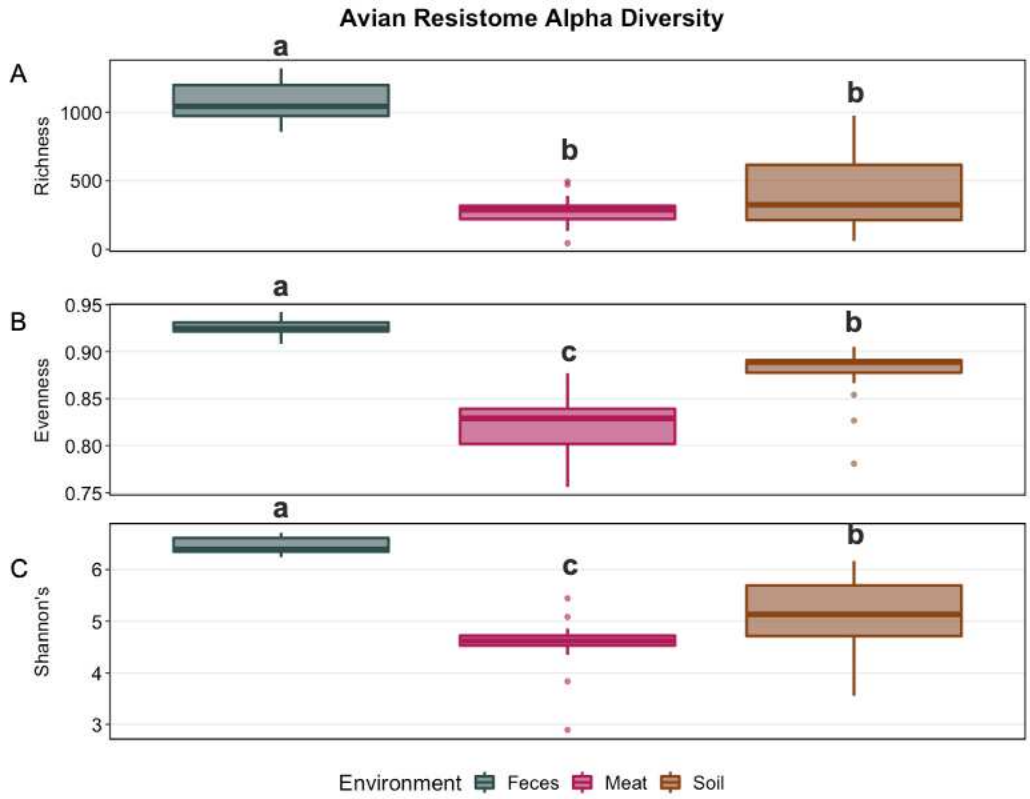


**Figure 3.1** The alpha diversity metrics of the resistome of avian associated environments. [abc] Means within an alpha diversity metric lacking a common superscript differ ($P < 0.05$). A) The richness, B) Pielou's evenness, and C) Shannon's alpha diversity index of the resistome of different avian environments.

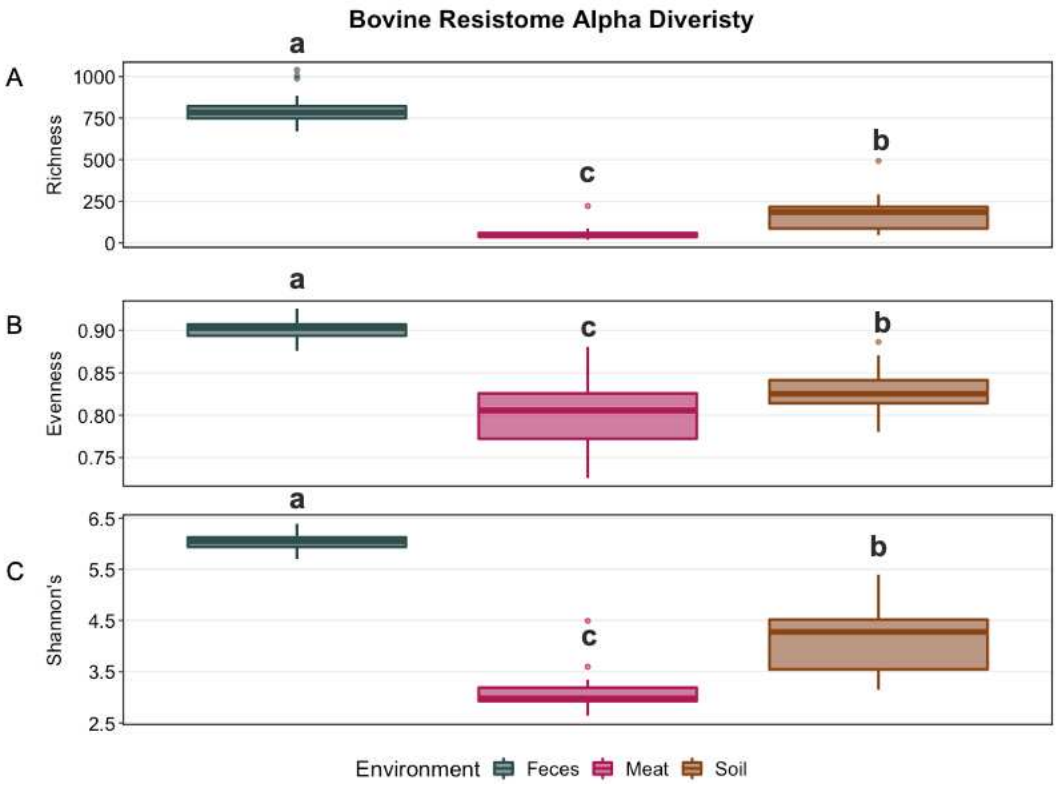


**Figure 3.2** The alpha diversity metrics of the resistome of bovine associated environments. [abc] Means within an alpha diversity metric lacking a common superscript differ ($P < 0.05$). A) The richness, B) Pielou's evenness, and C) Shannon's alpha diversity index of the resistome of different avian environments.

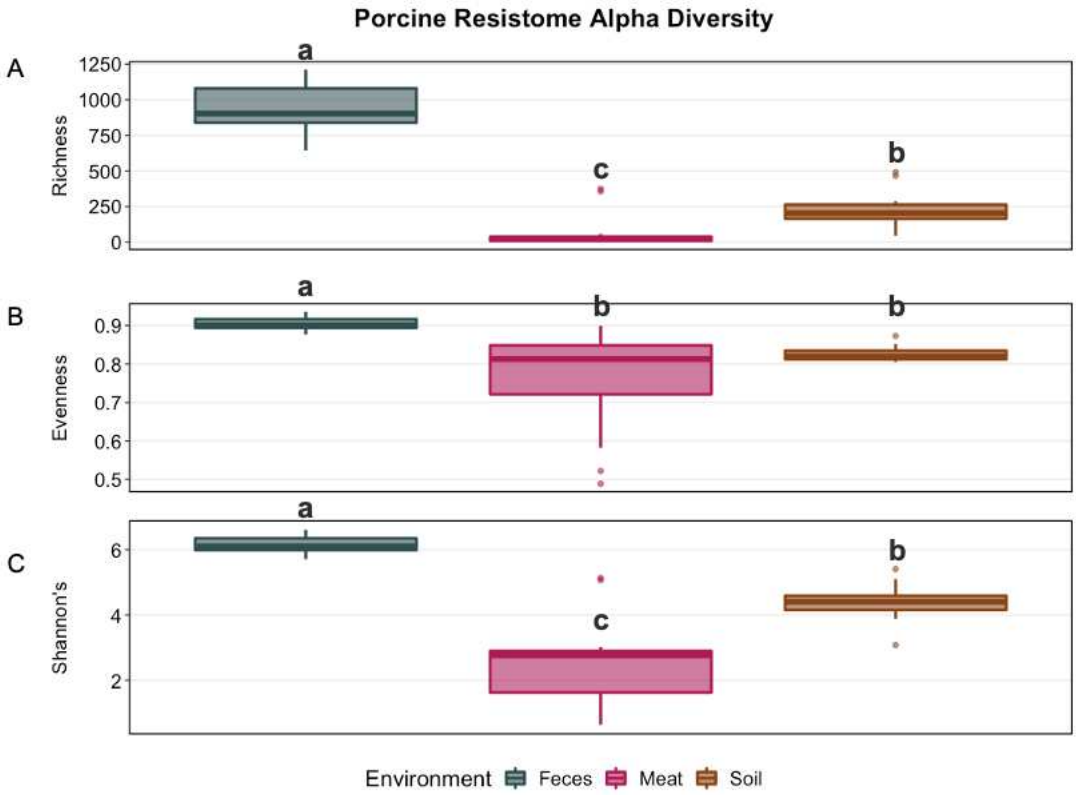


**Figure 3.3** The alpha diversity metrics of the resistome of porcine associated environments. [abc] Means within an alpha diversity metric lacking a common superscript differ ($P < 0.05$). A) The richness, B) Pielou's evenness, and C) Shannon's alpha diversity index of the resistome of different porcine environments.

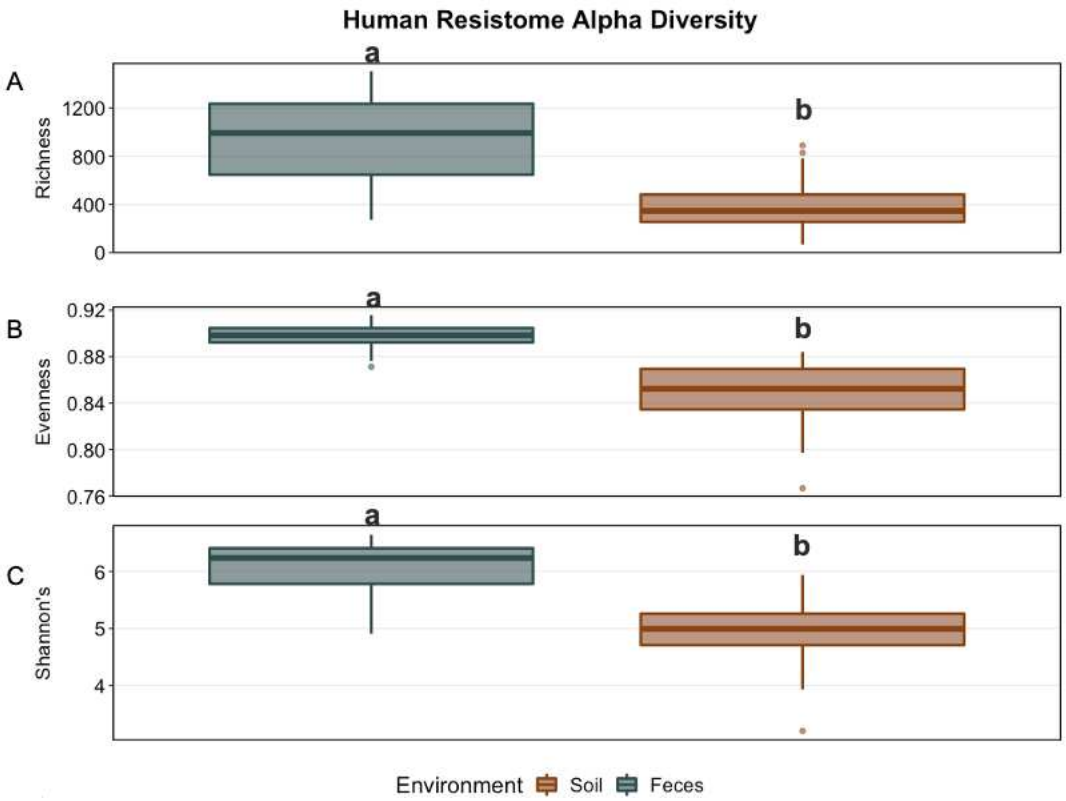


**Figure 3.4** The alpha diversity metrics of the resistome of human samples. [ab] Means within an alpha diversity metric lacking a common superscript differ ($P < 0.05$). A) The richness, B) Pielou's evenness, and C) Shannon's alpha diversity index of the resistome of different human samples.

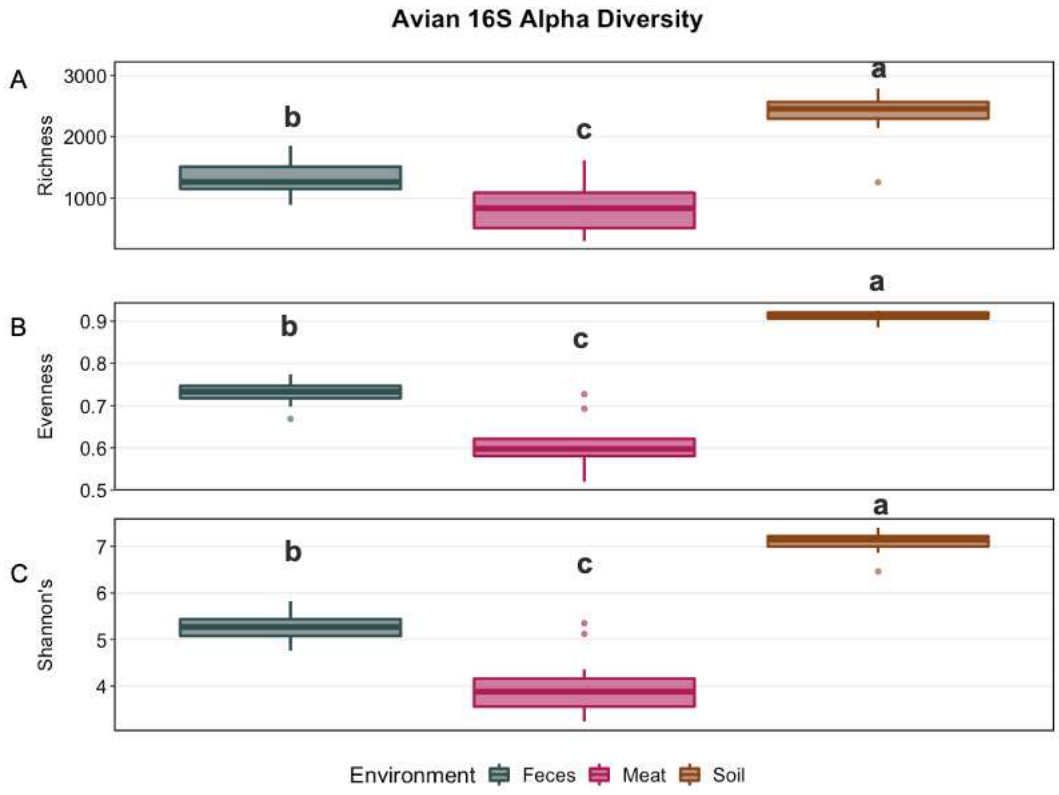


**Figure 3.5** The alpha diversity metrics of the microbiomes of avian associated environments. [abc] Means within an alpha diversity metric lacking a common superscript differ ($P < 0.05$). A) The richness, B) Pielou's evenness, and C) Shannon's alpha diversity index of the microbiomes of different avian environments.

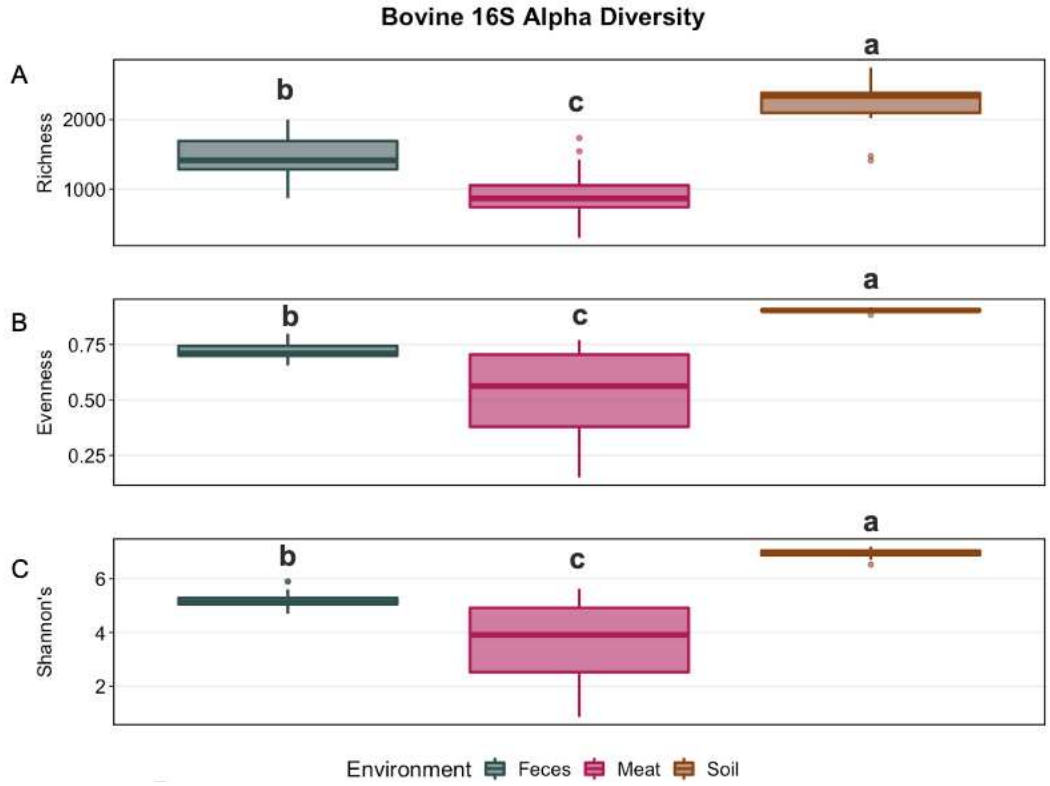


**Figure 3.6** The alpha diversity metrics of the microbiomes of bovine associated environments. [abc] Means within an alpha diversity metric lacking a common superscript differ ($P < 0.05$). A) The richness, B) Pielou's evenness, and C) Shannon's alpha diversity index of the microbiomes of different bovine environments.

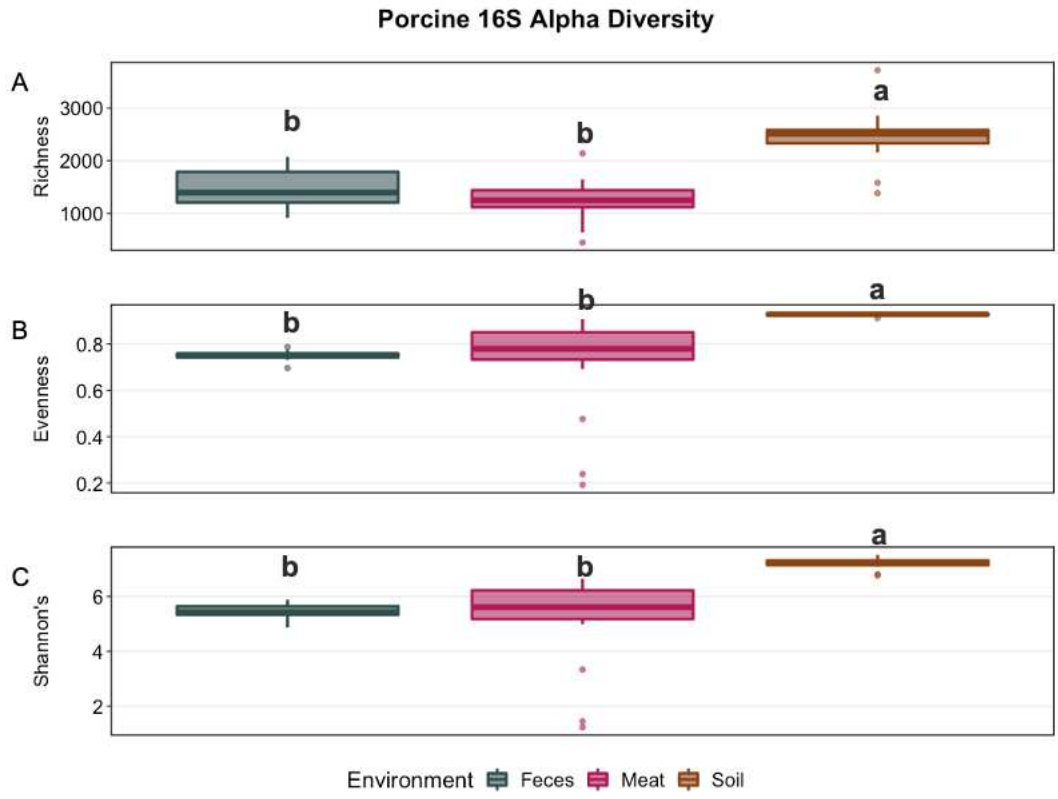


**Figure 3.7** The alpha diversity metrics of the microbiomes of porcine associated environments. [abc] Means within an alpha diversity metric lacking a common superscript differ ($P < 0.05$). A) The richness, B) Pielou's evenness, and C) Shannon's alpha diversity index of the microbiomes of different porcine environments.

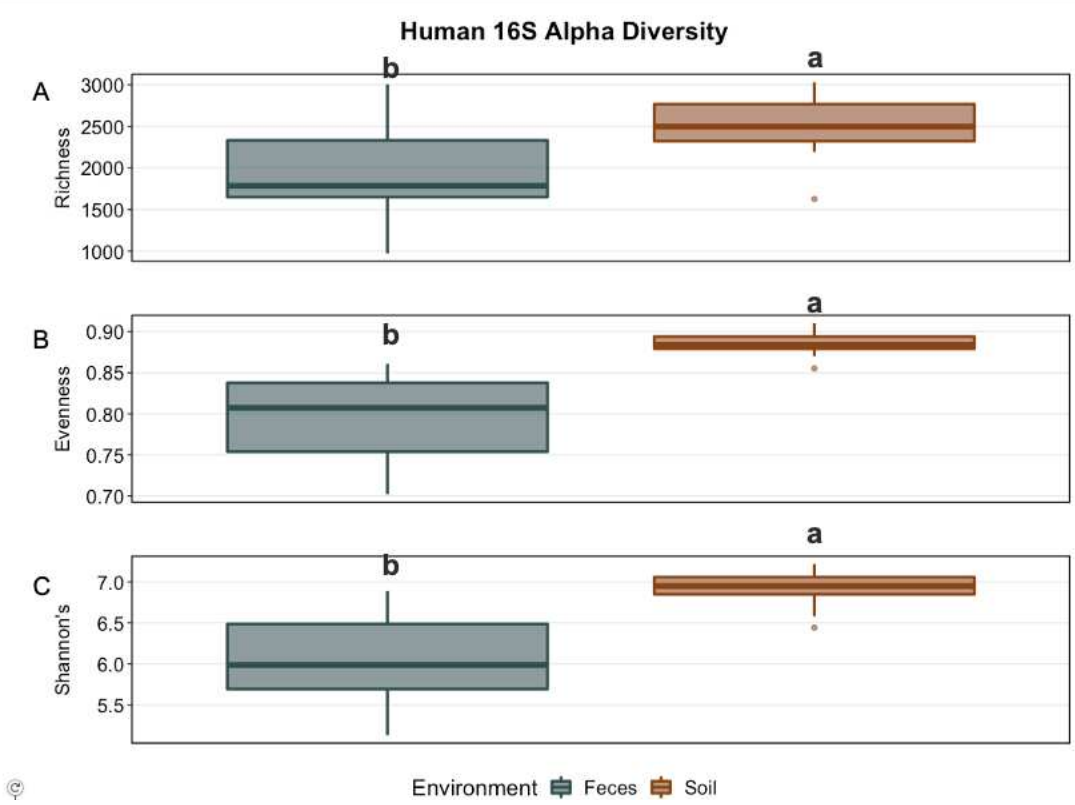


**Figure 3.8** The alpha diversity metrics of the microbiomes of human samples. [ab] Means within an alpha diversity metric lacking a common superscript differ ($P < 0.05$). A) The richness, B) Pielou's evenness, and C) Shannon's alpha diversity index of the microbiomes of human biosolid and human biosolid amending soil samples.

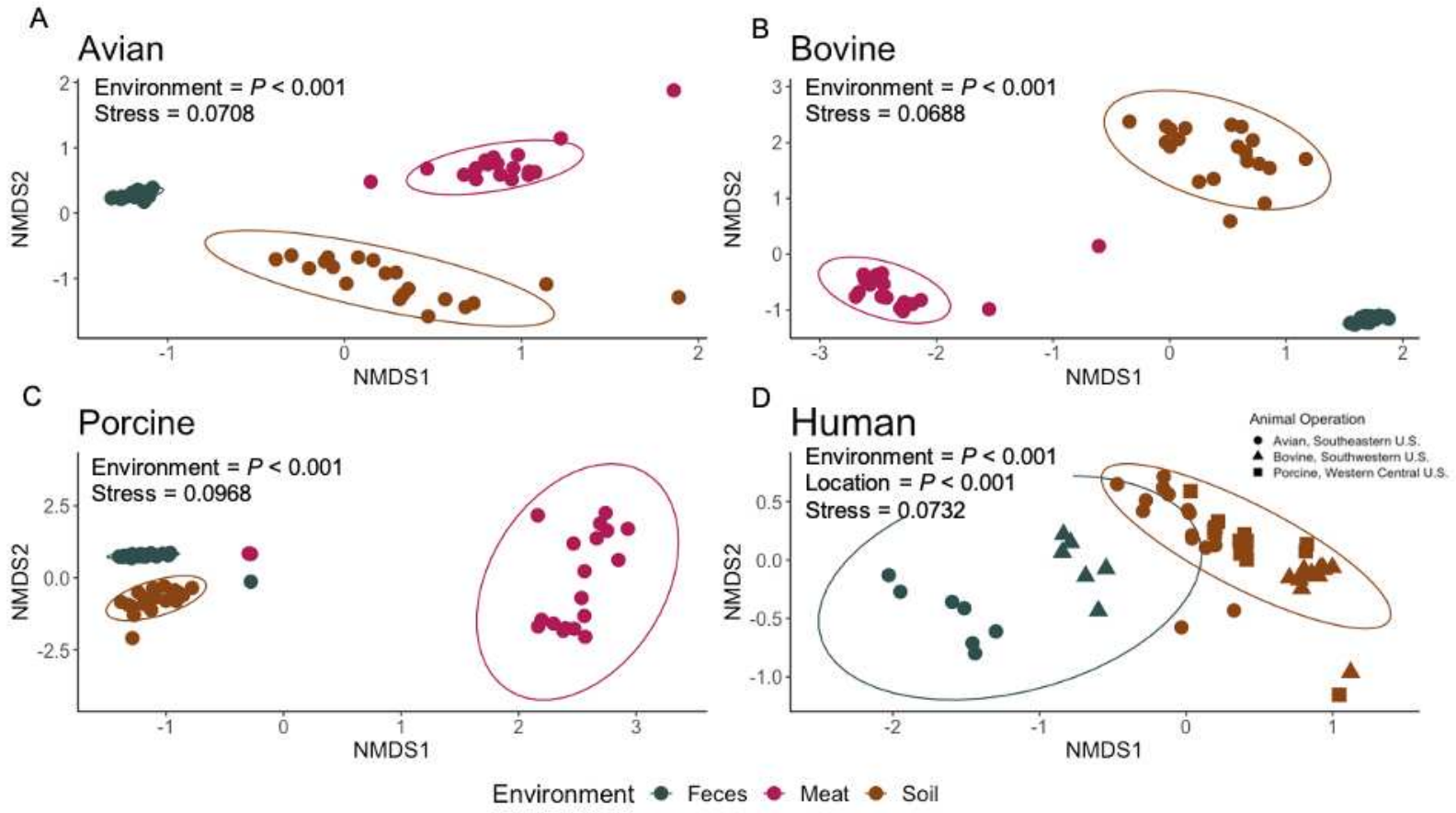


**Figure 3.9** Bray-Curtis dissimilarity ordination of the resistomes of avian, bovine, porcine, and human associated environments. A) The Bray-Curtis dissimilarity ordinations of the resistome of different avian associated environments. B.) The Bray-Curtis dissimilarity ordinations of the resistome of different bovine associated environments. C) The Bray-Curtis dissimilarity ordinations of the resistome of different porcine associated environments. D) The Bray-Curtis dissimilarity ordinations of the resistome of different human associated environments.

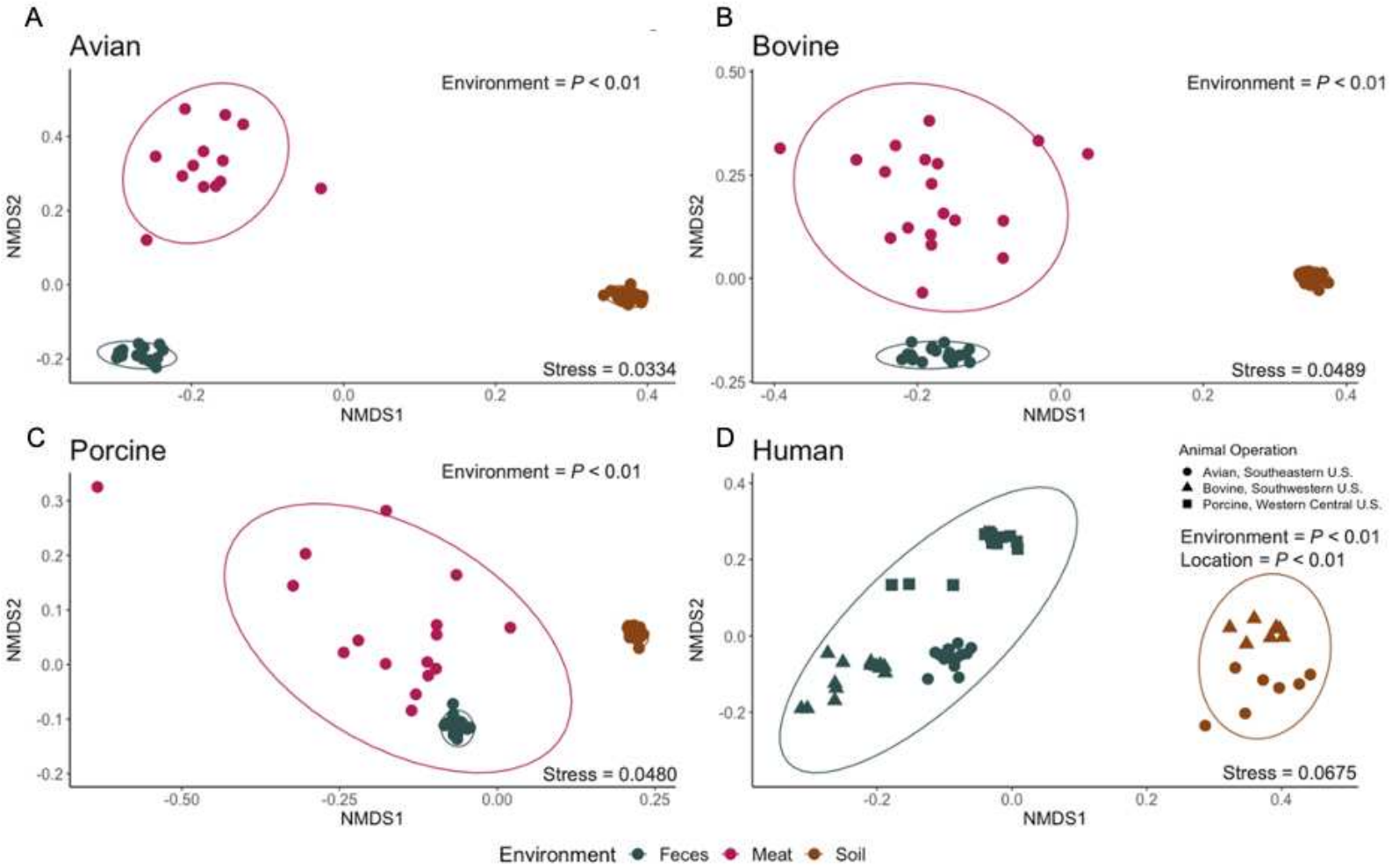


**Figure 3.10** Generalized Uni-Frac beta diversity ordination of the microbiomes of avian, bovine, porcine, and human associated environments**.** A) Generalized Uni-Frac beta diversity ordinations of the microbiome of different avian associated environments. B) Generalized Uni-Frac beta diversity ordinations of the microbiome of different bovine associated environments. C) Generalized Uni-Frac beta diversity ordinations of the microbiome of different associated environments. D) Generalized Uni-Frac beta diversity ordinations of the microbiome of different human associated environments.

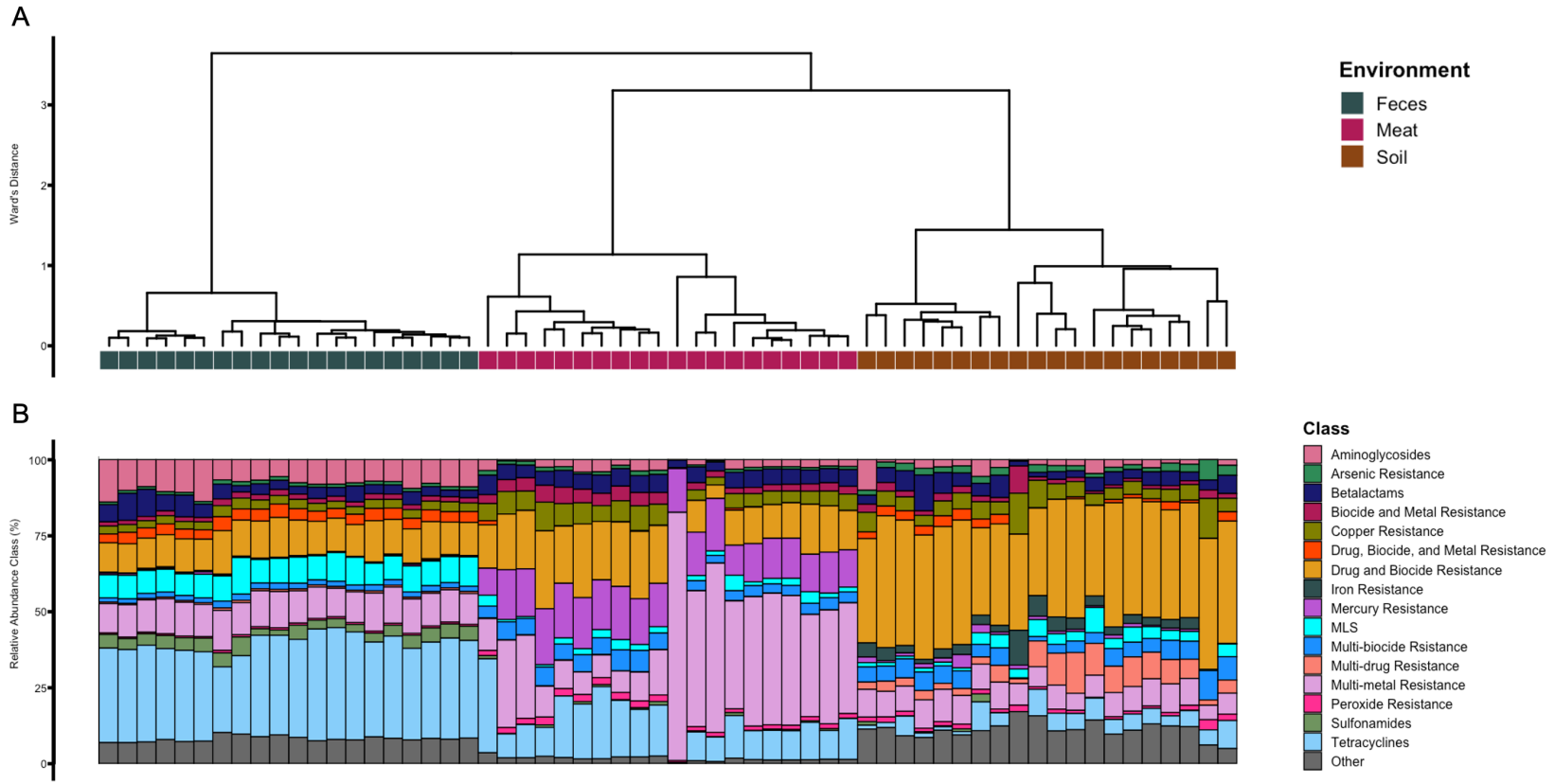


**Figure 3.11** Ward's Hierarchal Clustering and relative abundance of the resistome of avian samples. A) Ward's Hierarchal Clustering of the resistome of different avian associated environments. B) The relative abundance of antimicrobial resistance at the class level for the corresponding avian samples. Classes of Resistance are colored by class groupings and the 16 most abundant classes are displayed in the legend.

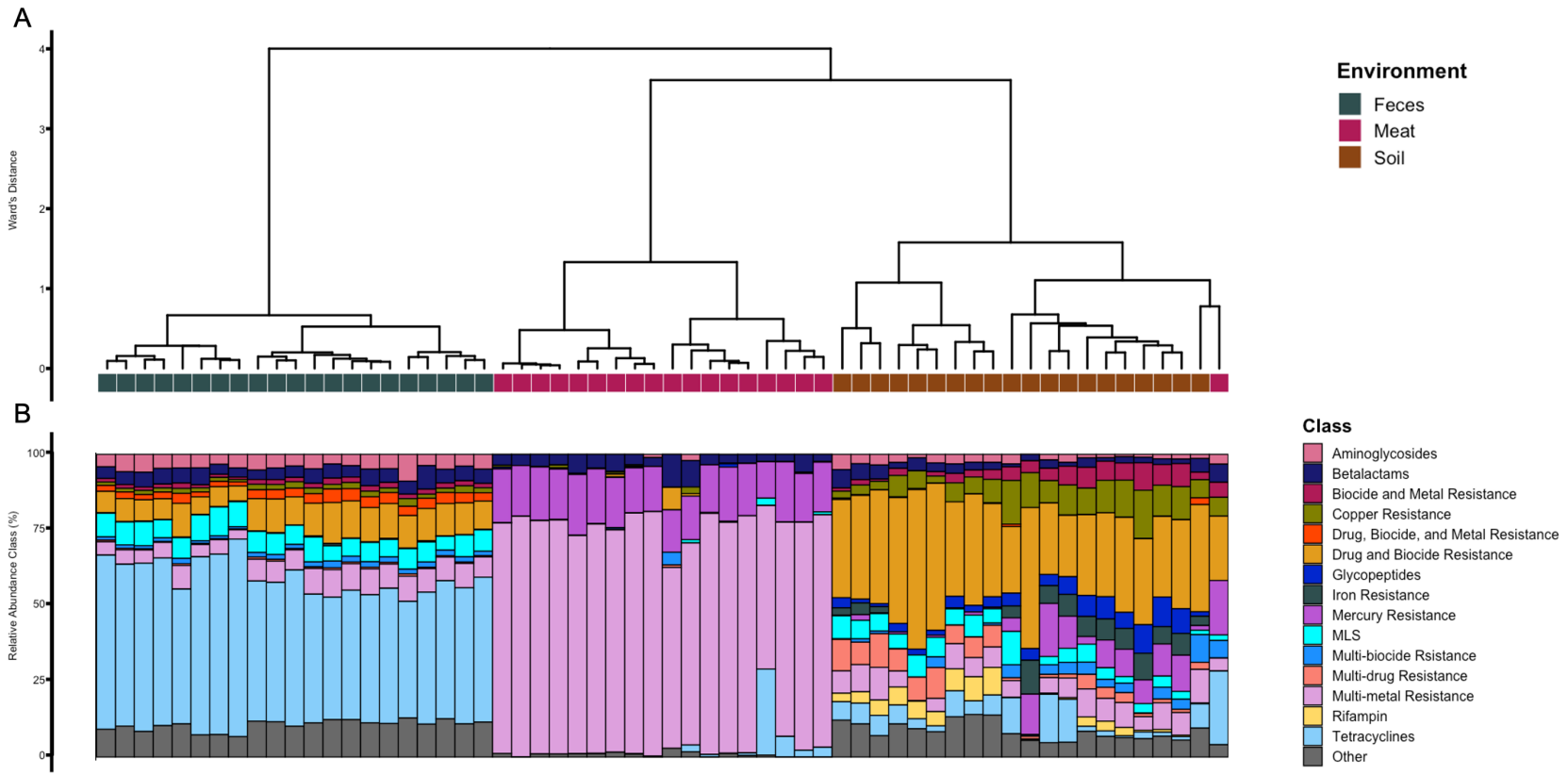


**Figure 3.12** Ward's Hierarchal Clustering and relative abundance of the resistome of bovine samples. A) Ward's Hierarchal Clustering of the resistome of different bovine associated environments. B) The relative abundance of antimicrobial resistance at the class level for the corresponding bovine samples. Classes of Resistance are colored by class groupings and the 16 most abundant classes are displayed in the legend.

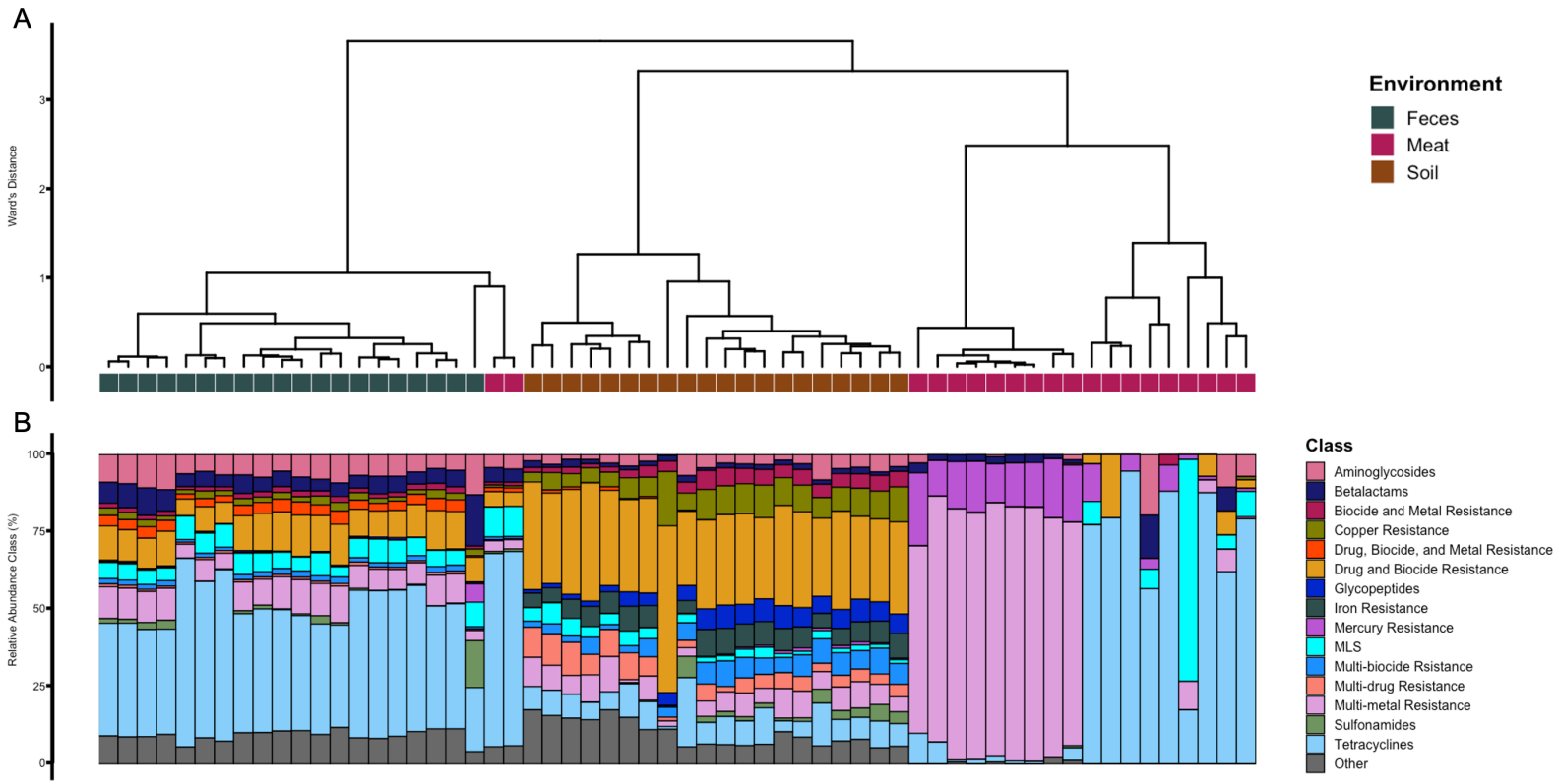


**Figure 3.13** Ward's Hierarchal Clustering and relative abundance of the resistome of porcine samples. A.) Ward's Hierarchal Clustering of the resistome of different porcine associated environments. B.) The relative abundance of antimicrobial resistance at the class level for the corresponding porcine samples. Classes of Resistance are colored by class groupings and the 16 most abundant classes are displayed in the legend.

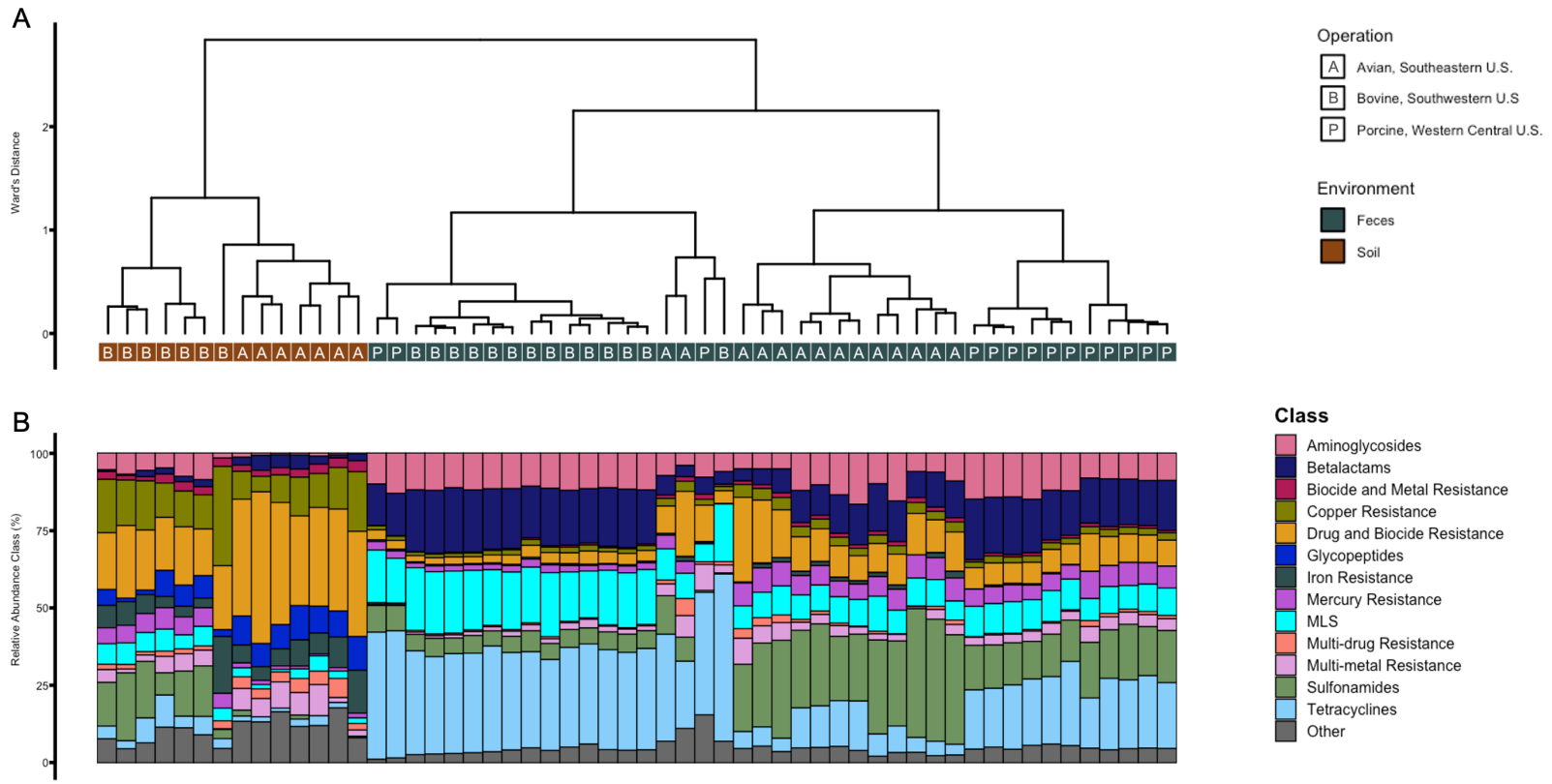


**Figure 3.14** Ward's Hierarchal Clustering and relative abundance of the resistome of human samples. A.) Ward's Hierarchal Clustering of the resistome of different human samples. B.) The relative abundance of antimicrobial resistance at the class level for the corresponding human samples. Classes of Resistance are colored by class groupings and the 16 most abundant classes are displayed in the legend.

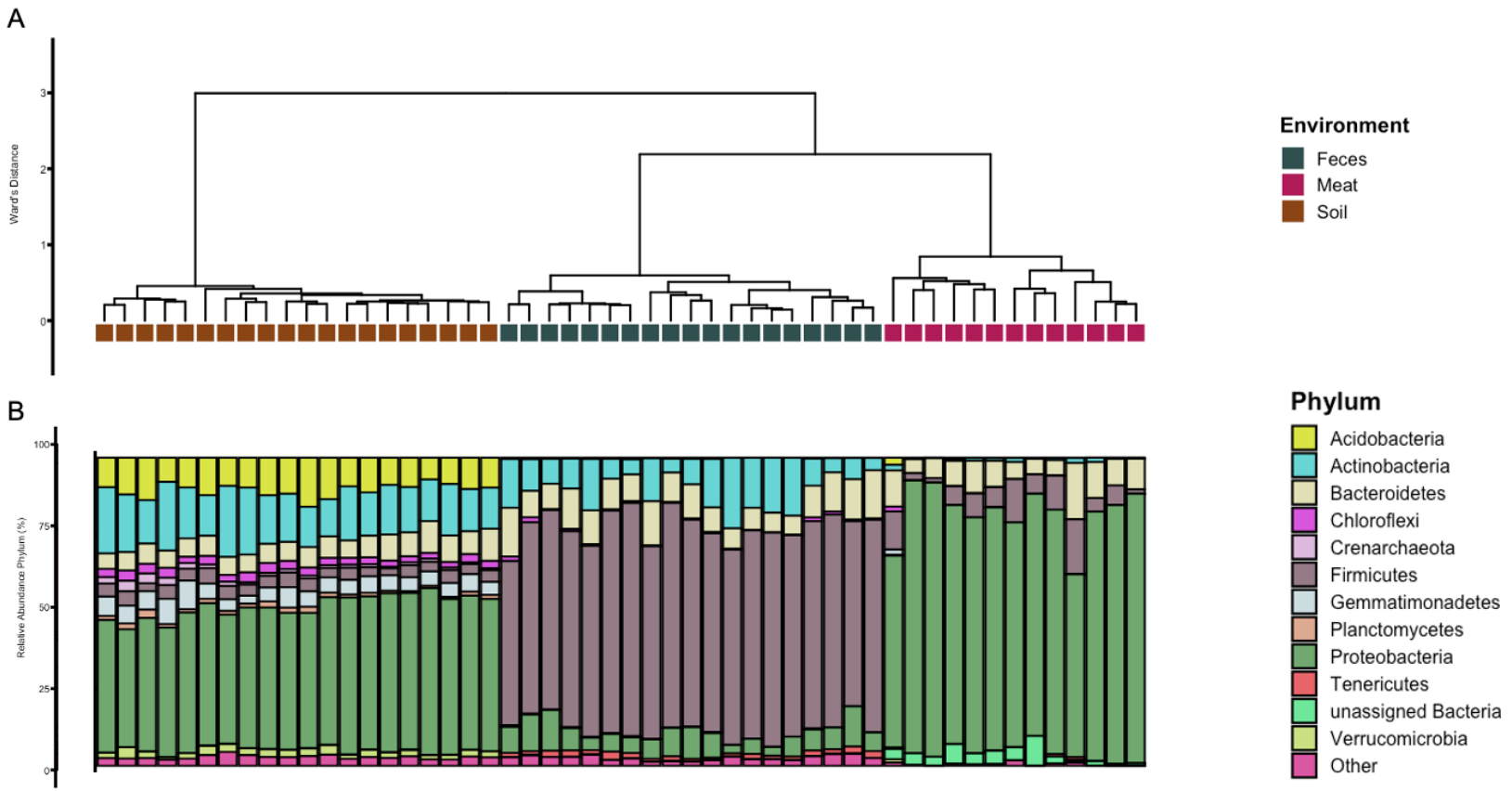


**Figure 3.15** Ward's Hierarchal Clustering and relative abundance of the microbiome of avian samples. A.) Ward's Hierarchal Clustering of the microbiome of different avian associated environments. B.) The relative abundance of microbiome at the phylum level for the corresponding avian samples.

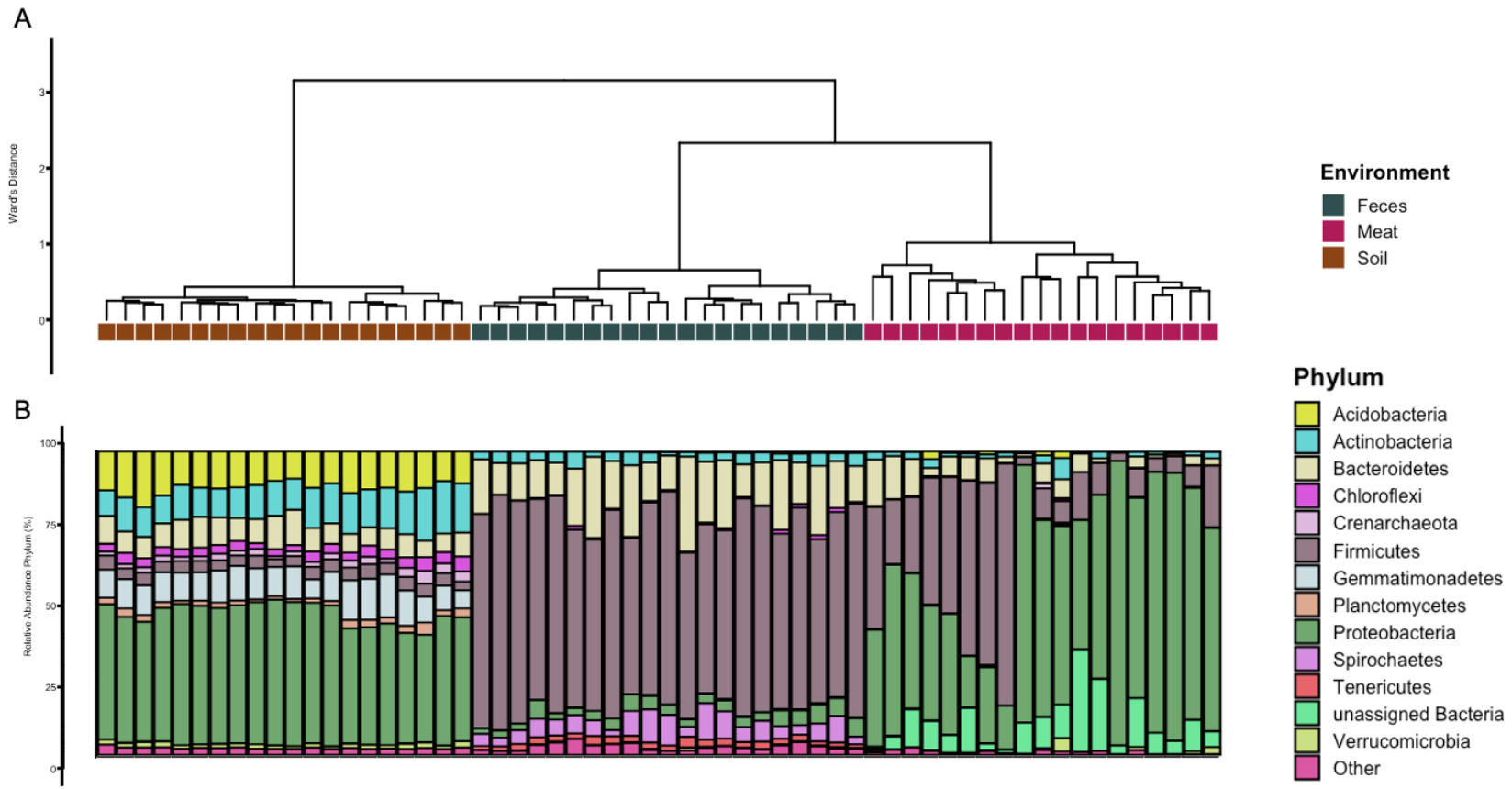


**Figure 3.16** Ward's Hierarchal Clustering and relative abundance of the microbiome of bovine samples. A.) Ward's Hierarchal Clustering of the microbiome of different bovine associated environments. B.) The relative abundance of microbiome at the phylum level for the corresponding bovine samples.

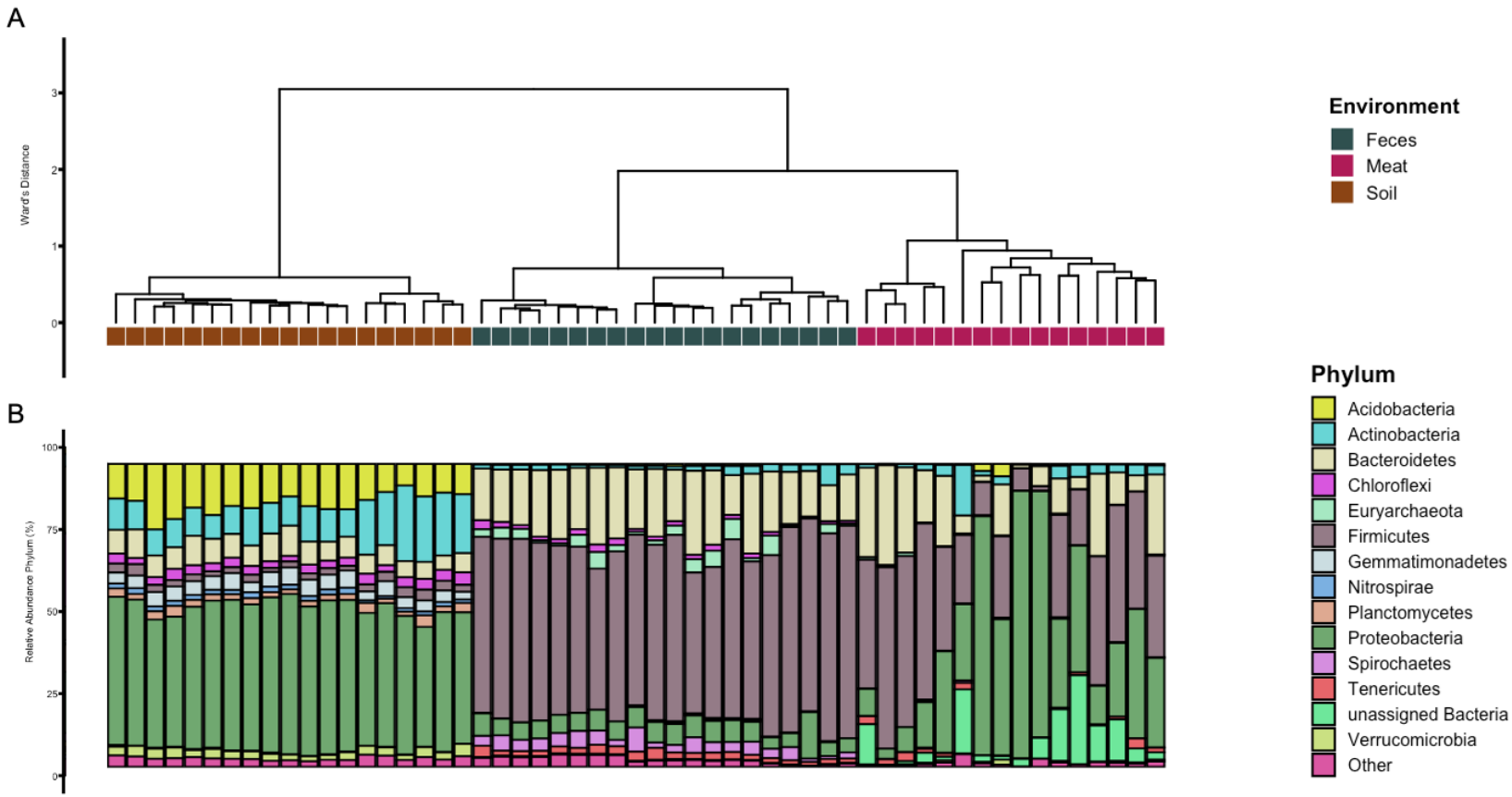


**Figure 3.17** Ward's Hierarchal Clustering and relative abundance of the microbiome of porcine samples. A.) Ward's Hierarchal Clustering of the microbiome of different porcine associated environments. B.) The relative abundance of microbiome at the phylum level for the corresponding porcine samples.

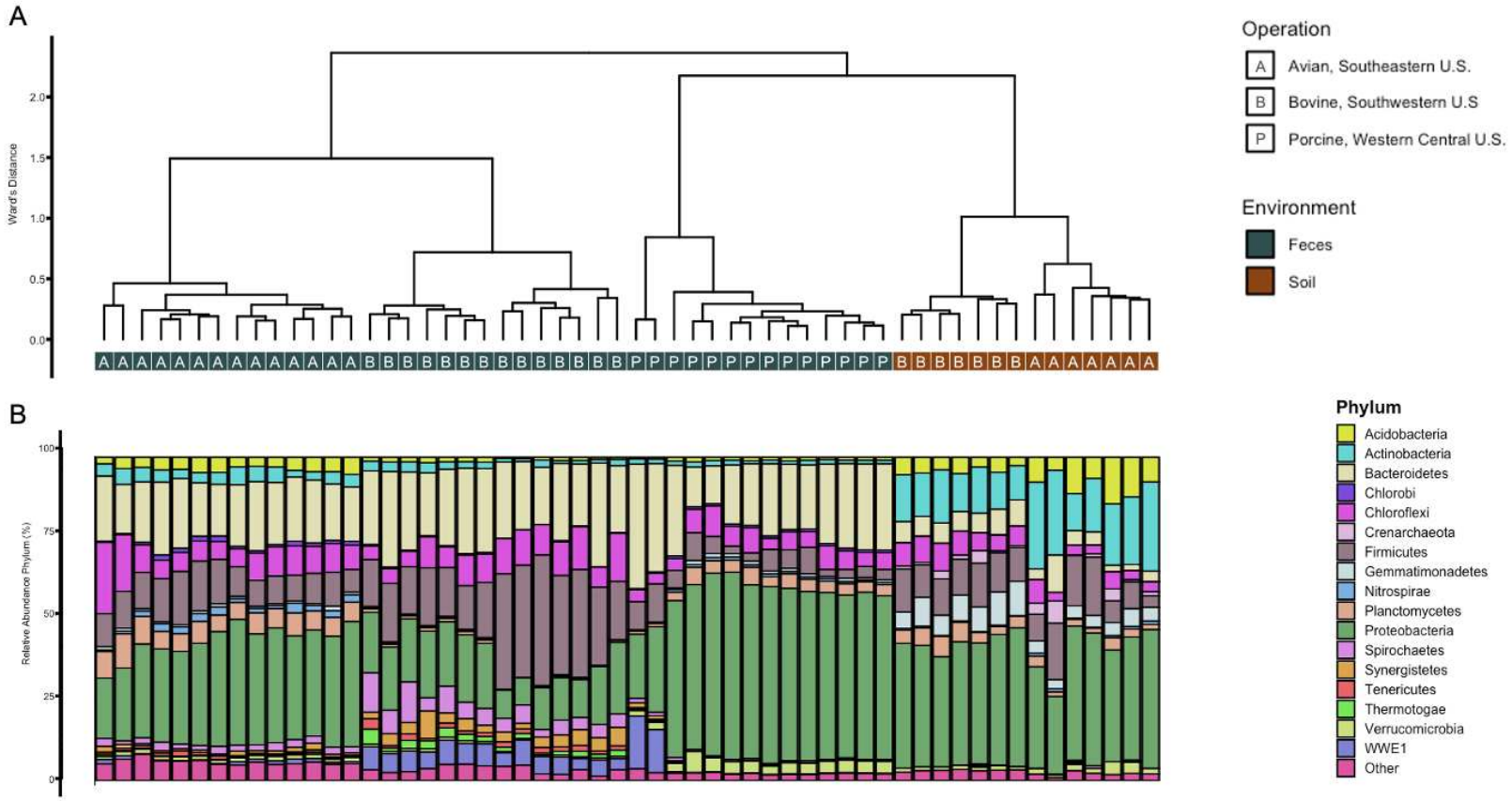


**Figure 3.18** Ward's Hierarchal Clustering and relative abundance of the microbiome of human samples. A.) .) Ward's Hierarchal Clustering of the microbiome of different human samples. B The relative abundance of microbiome at the phylum level for the corresponding human samples. Classes of Resistance are colored by class groupings and the 16 most abundant classes are displayed in the legend.

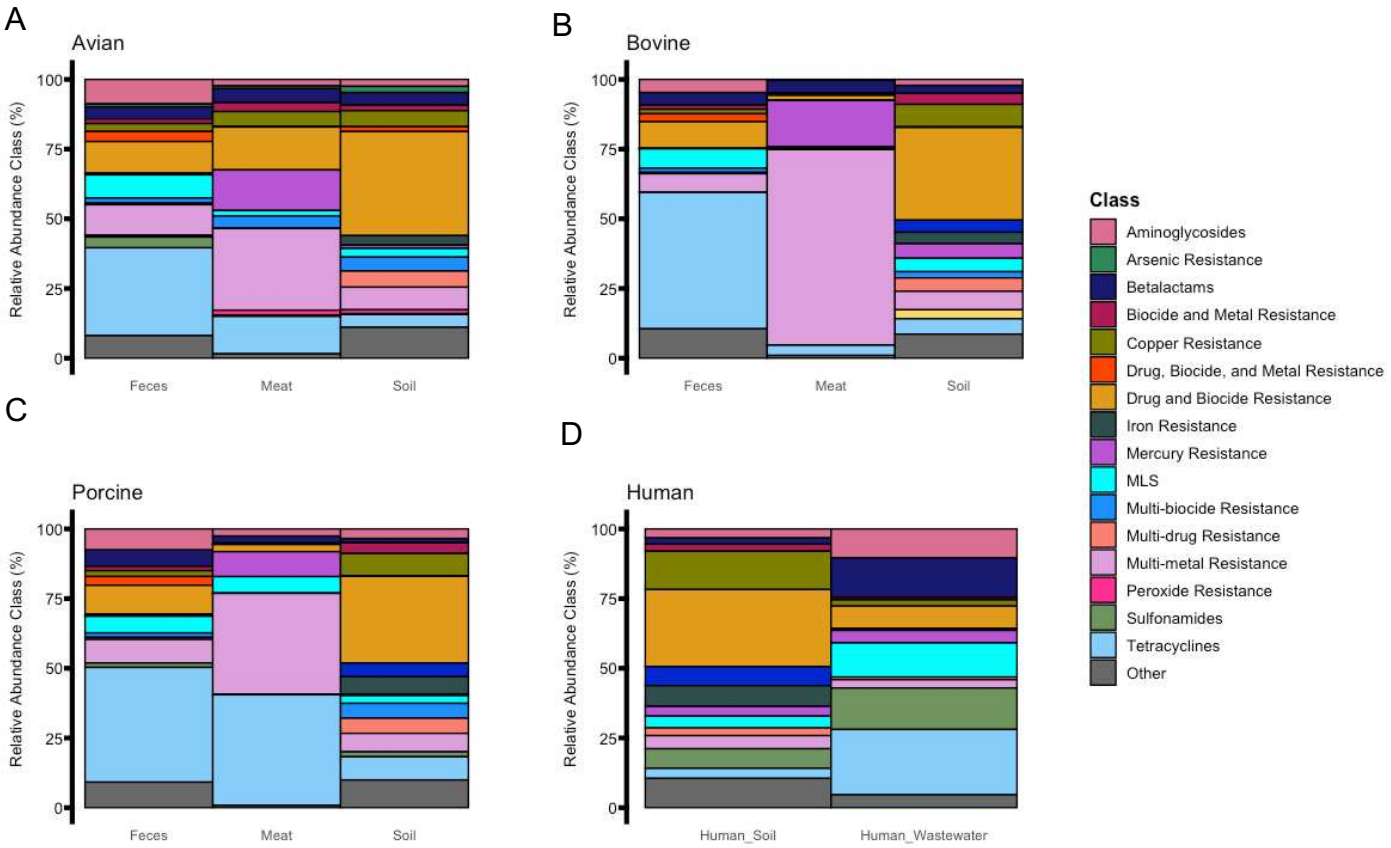


**Figure 3.19** The relative abundance of antimicrobial resistance at the class level for avian, bovine, porcine, and human associated environments. Classes of Resistance are colored by class groupings and the 16 most abundant classes are displayed in the legend. A.) The relative abundance of resistance classes of different avian associated environments. B.) The relative abundance of resistance classes of different bovine associated environments. C.) The relative abundance of resistance classes of different porcine associated environments. D.) The relative abundance of resistance classes of different human associated environments.

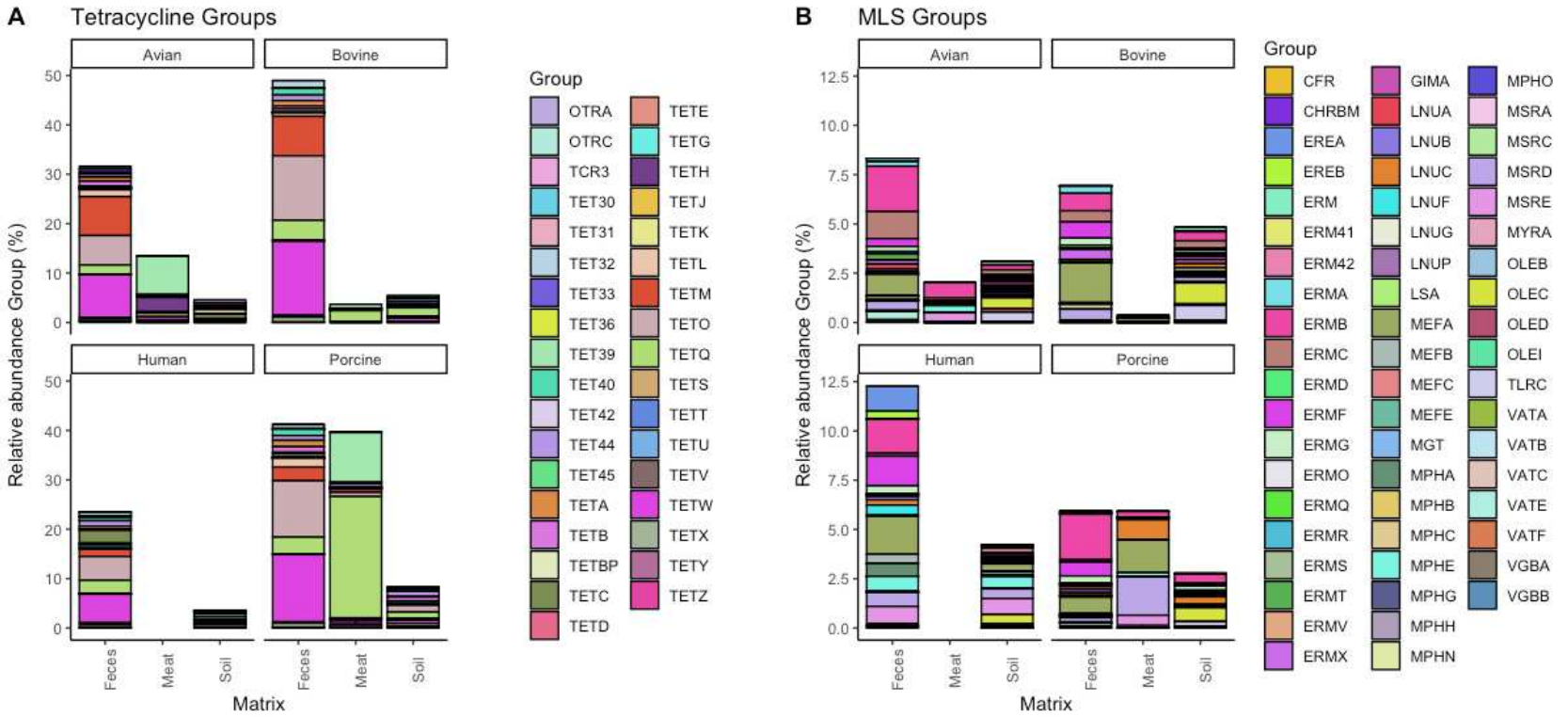


**Figure 3.20** The relative abundance of antimicrobial resistance gene groups for avian, bovine, porcine, and human associated environments. Genes are colored by group displayed in the legend. A.) The relative abundance of resistance classes of different avian associated environments. A.) Antimicrobial resistance genes specific to tetracyclines in avian bovine, porcine, and human environments. B.) Antimicrobial resistance genes specific to macrolides, lincosamides, and streptogramins (MLS) in avian bovine, porcine, and human environments.

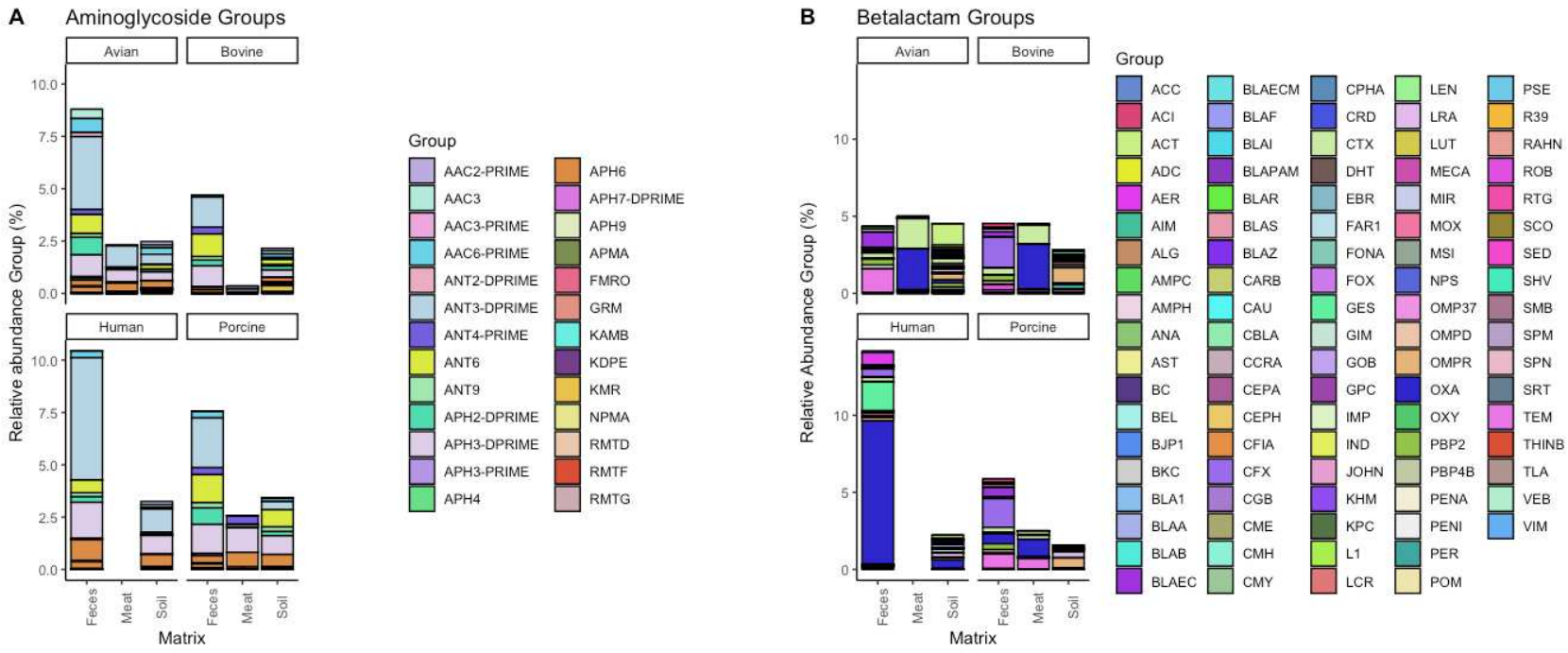


**Figure 3.21**The relative abundance of antimicrobial resistance gene groups for avian, bovine, porcine, and human associated environments. Genes are colored by group displayed in the legend. A.) The relative abundance of resistance classes of different avian associated environments. A.) Antimicrobial resistance genes specific to Aminoglycosides in avian bovine, porcine, and human environments. B.) Antimicrobial resistance genes specific to betalactams in avian bovine, porcine, and human environments.

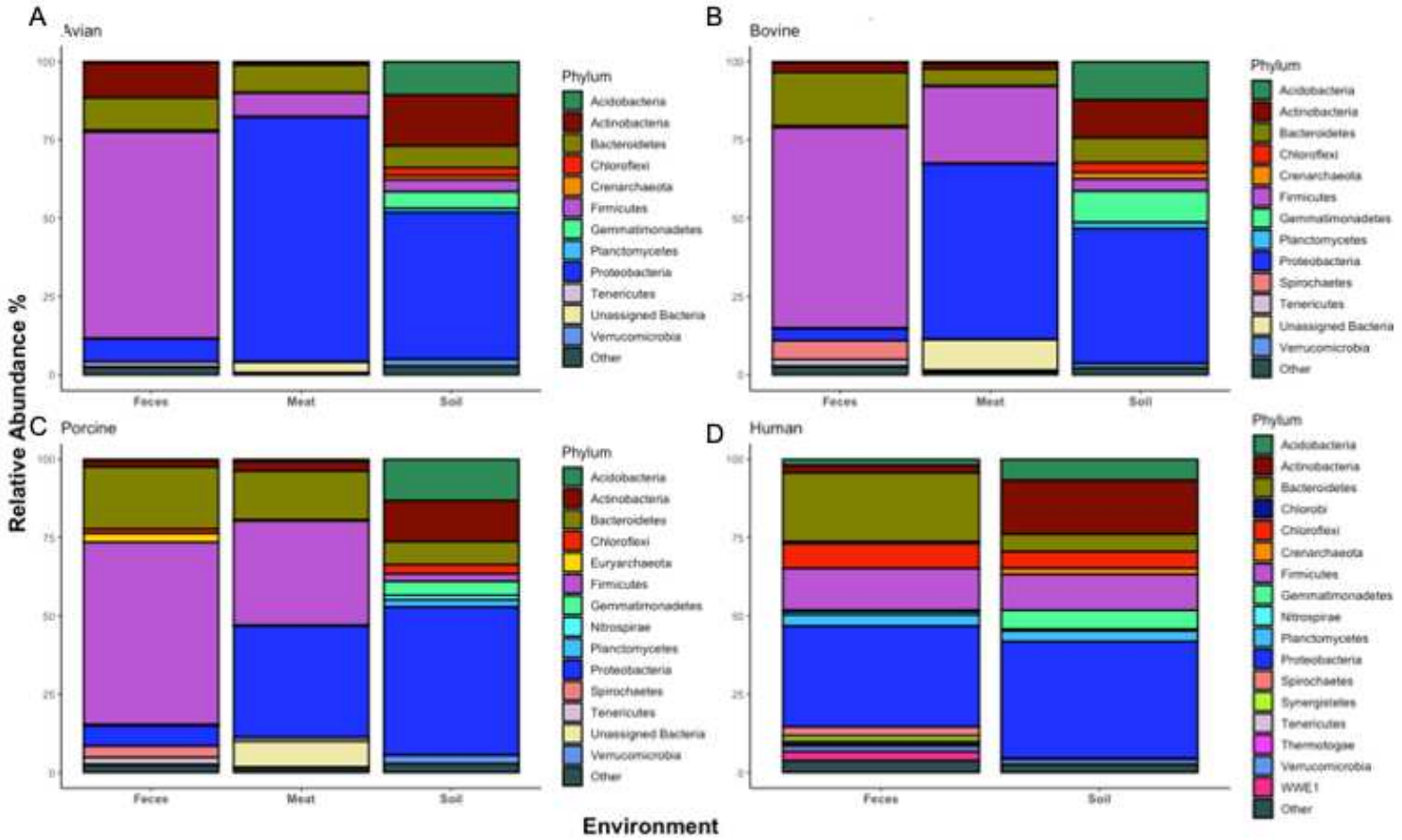


**Figure 3.22** The relative abundance of the microbiome at the phylum level for avian, bovine, porcine, and human associated environments. different colors represent different phylums in the legend. A.) The relative abundance at the phylum level of different avian associated environments. B.) The relative abundance at the phylum level of different bovine associated environments. C.) The relative abundance at the phylum level of different porcine associated environments. D.) The relative abundance at the phylum level of different human associated environments.

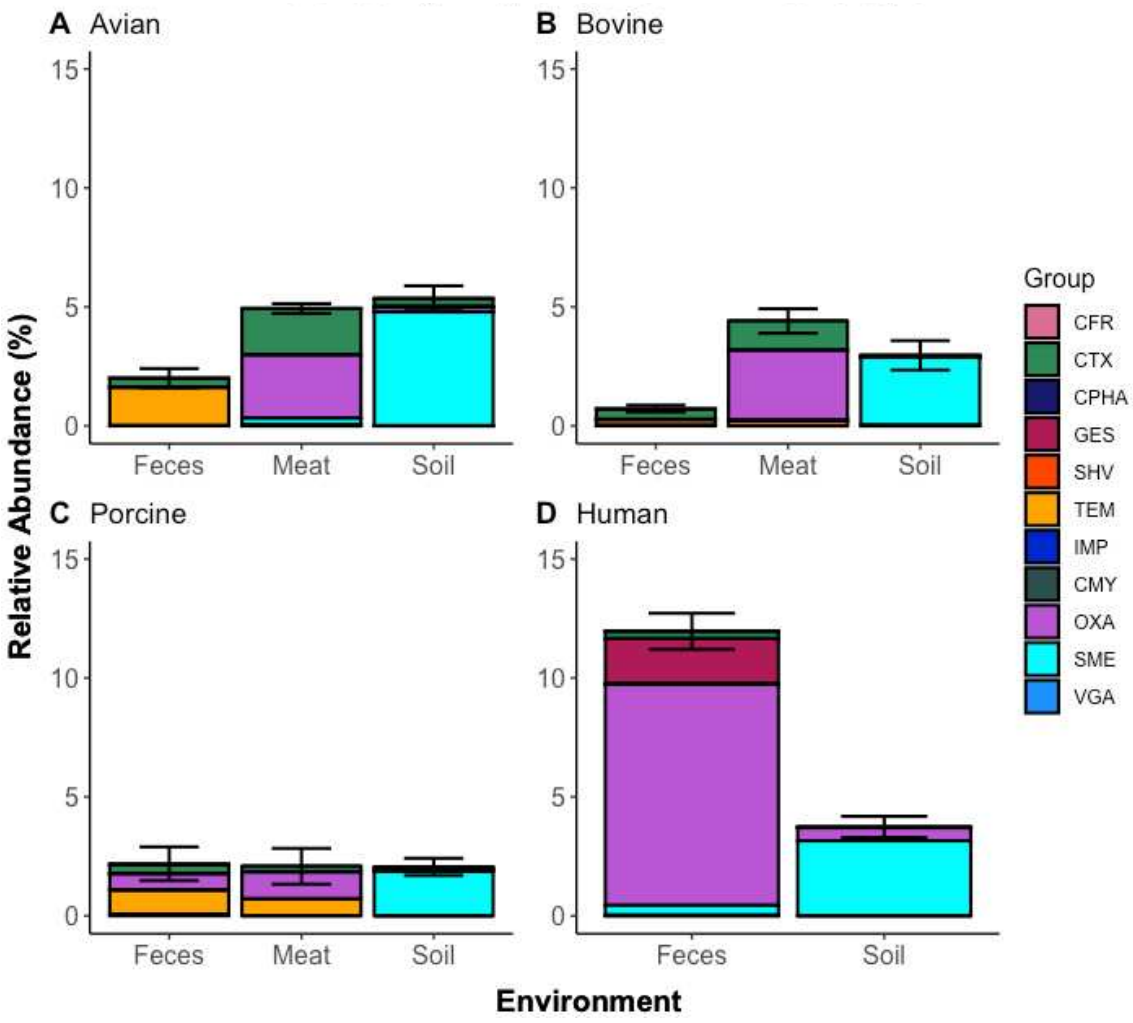


**Figure 3.23** The relative abundance of medically important antimicrobial resistance genes for avian, bovine, porcine, and human associated environments. Classes of Resistance are colored by group in the legend. A.) The relative abundance of medically important resistance genes of different avian associated environments. B.) The relative abundance of medically important resistance genes of different bovine associated environments. C.) The relative abundance of medically important resistance genes of different porcine associated environments. D.) The relative abundance of medically important resistance genes of different human associated environments.

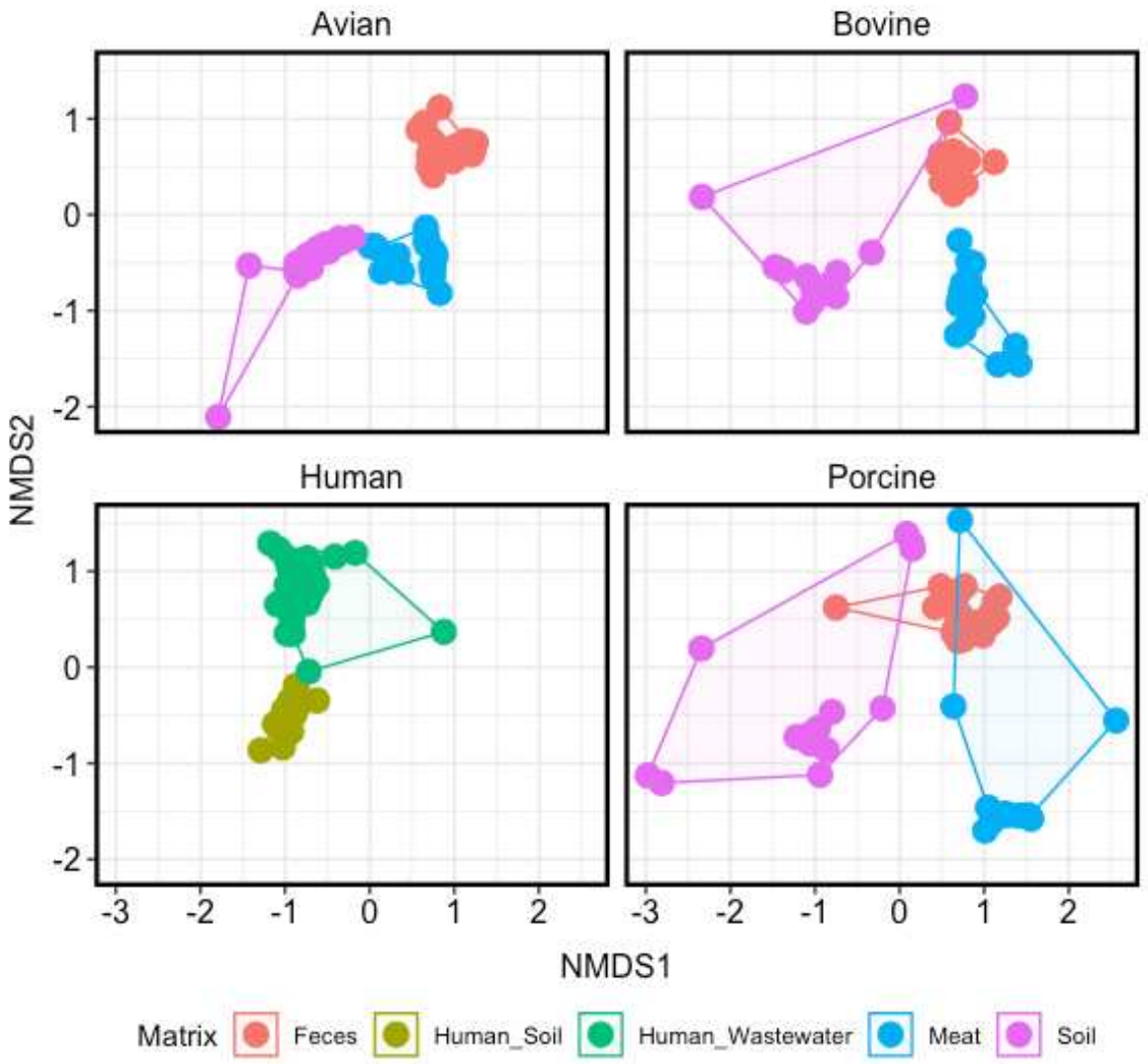


**Figure 3.24** Bray-Curtis dissimilarity ordination of medically important resistance genes of avian, bovine, porcine, and human associated environments. A.) The Bray-Curtis dissimilarity ordinations of medically important resistance genes of different avian associated environments. B.) The Bray-Curtis dissimilarity ordinations of medically important resistance genes of different bovine associated environments. C.) The Bray-Curtis dissimilarity ordinations of medically important resistance genes of different porcine associated environments. D.) The Bray-Curtis dissimilarity ordinations of medically important resistance genes of different human associated environments.

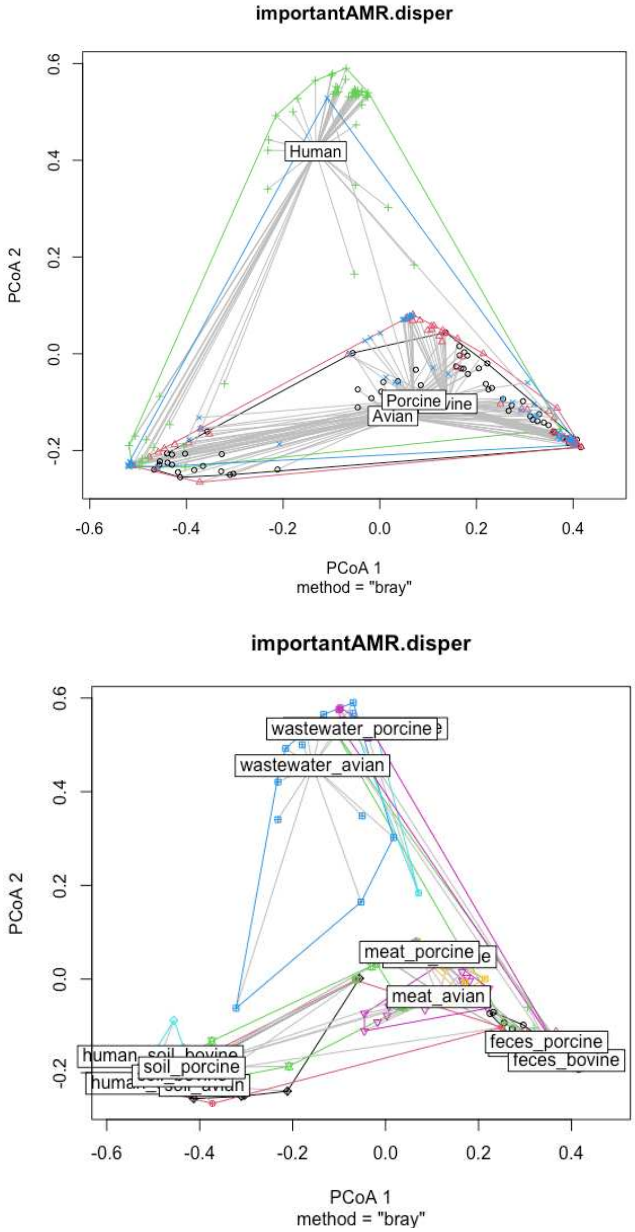


**Figure 3.25** Multivariate analogue of Levene's test for homogeneity of variances for all medically important antimicrobial resistance genes. A.) Multivariate analogue of Levene's test for homogeneity of variances for all medically important antimicrobial resistance genes by host (avian, bovine, porcine, human) for all environments (feces, meat, soil). B.) Multivariate analogue of Levene's test for homogeneity of variances for all medically important antimicrobial resistance genes by host (avian, bovine, porcine, human) and environment (feces, meat, and soil)

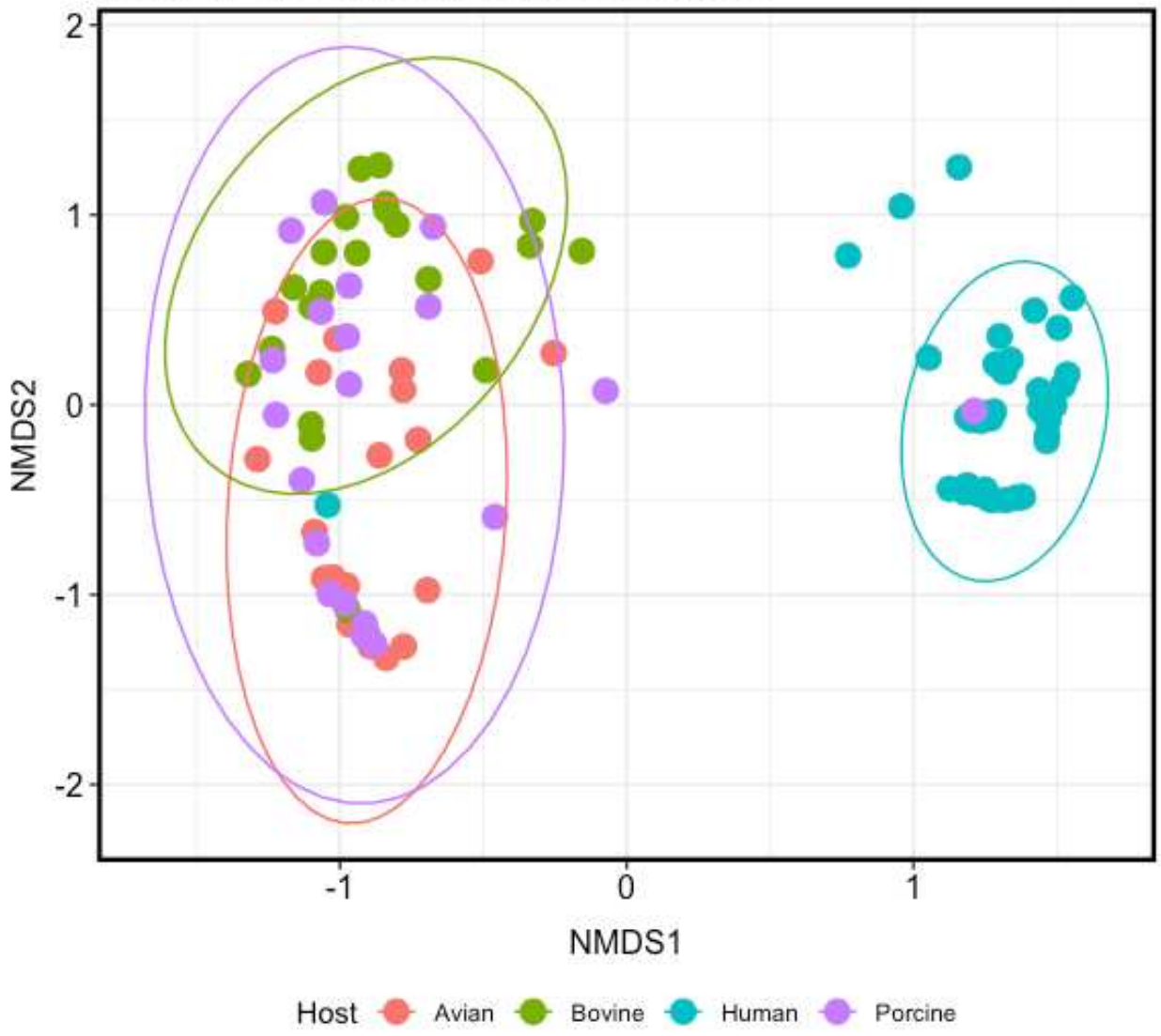


**Figure 3.26** Bray-Curtis dissimilarity ordination of medically important resistance genes of avian, bovine, porcine, and human feces and biosolid samples.

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