

# In-depth Analysis of Seafood Resistome using Shotgun Metagenomics

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## Abstract

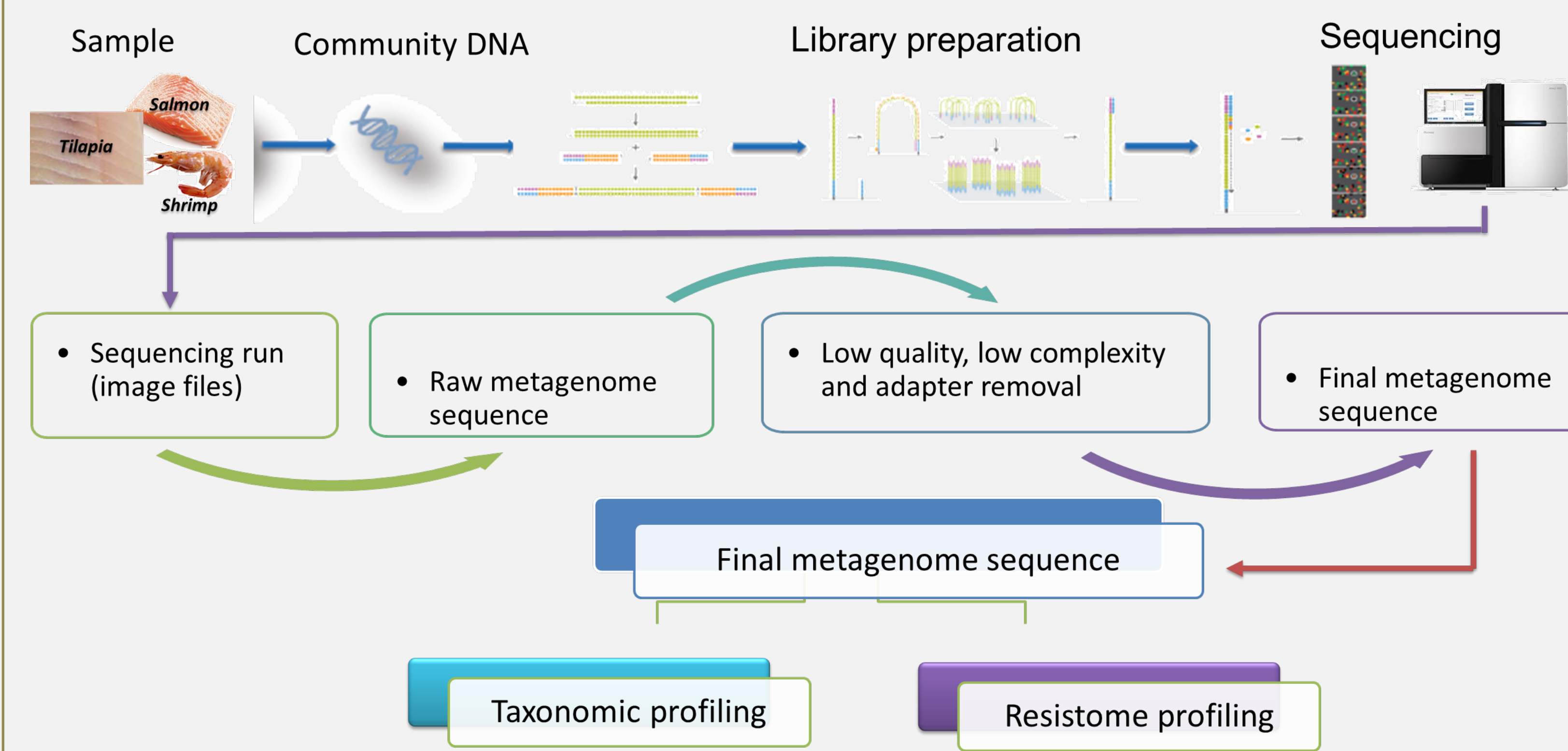
Background: Comprehensive profiling of antibiotic resistance genes in the farm to fork food supply chain is a goal of integrated surveillance programs. The distribution and relative abundances of antimicrobial resistance genes (ARGs) in seafood microbiomes are not well understood. High-throughput next generation sequencing technologies offer new approaches for antimicrobial resistance monitoring. In this study, we employed a shotgun metagenomics approach to catalogue and quantify resistance genes present in different seafood samples collected by the National Antimicrobial Resistance Monitoring System. Methods: A total of 122 seafood samples (41 salmon, 35 shrimp and 46 tilapia) were included in the study. Community DNA was extracted and sequenced on a HiSeq2500 sequencer using 2x125 bp paired-end sequencing. The presence and abundance of ARGs in the metagenomic dataset were determined using the Short, Better Representative Extract Dataset (ShortBRED) unique peptide markers generated from the AMRFinderPlus protein database. We used LDA Effect Size (LEfSe) to determine the resistance genes most likely to explain the difference between sources. Results: We identified 91 resistance genes representing 15 antimicrobial resistance classes including  $\beta$ -Lactam, aminoglycosides, tetracycline, quinolone, macrolide, and phenicol. We identified more than 31  $\beta$ -Lactam resistance genes including *bla<sub>OXA</sub>*, *bla<sub>IMP</sub>*, *bla<sub>CMY</sub>* and *bla<sub>FOX</sub>* genes in our study. In addition, we detected fluoroquinolone resistance genes namely: *qnrA*, *qnrB*, *qnrD*, *qnrS*, *qnrVC* and *oqxB* genes. The distribution and relative abundance of ARGs observed varied by sample type. We found that salmon samples contained 73 ARGs, tilapia 74 ARGs and shrimp samples 60 ARGs. The three most common antimicrobial resistance genes observed in salmon microbiomes were *aac(3)-VIIa* (70.7%), *bla<sub>LRA-1</sub>* (53.7%), *emhC* (46.3%) and *tet(L)* (46.3%) while *aac(3)-VIIa* (68.6%) was the most common AR gene in shrimp followed by *emhC* (51.4%) and *bla<sub>OXA-548</sub>* family (48.6%). Among tilapia samples, *aac(3)-VIIa* (58.7%) and *bla<sub>OXA-548</sub>* family (58.7%) were the most common ARGs followed by *tet(L)* (56.5%). Conclusions: Our study provides valuable insights into the diversity and identity of resistance genes present in seafood samples. This work helps characterize the resistome of seafood microbiome beyond what can be determined by culture-based methods alone. This information will help shed light on the potential of metagenomics as an auxiliary method for monitoring resistance in the food supply.

## Background

Comprehensive profiling of antibiotic resistance genes available from farm to fork continuum are essential to understand the resistance potential for entire microbial communities. The distribution and relative abundances of antibiotic resistance (AR) genes in seafood microbiomes are not well understood. Since antibiotic resistance determinants are readily exchanged between bacterial species, there is an increasing interest in investigating reservoirs of antibiotic resistance accessible to pathogens along the 'farm to fork' continuum. In this study, we employed a shotgun metagenomics approach to catalogue and quantify resistance genes present in different seafood samples collected by the National Antimicrobial Resistance Monitoring System.

## Approach

Figure 1. Metagenomics workflow: sample processing to data analysis



## Results

Figure 2. Bubble graph showing the major antimicrobial resistance classes

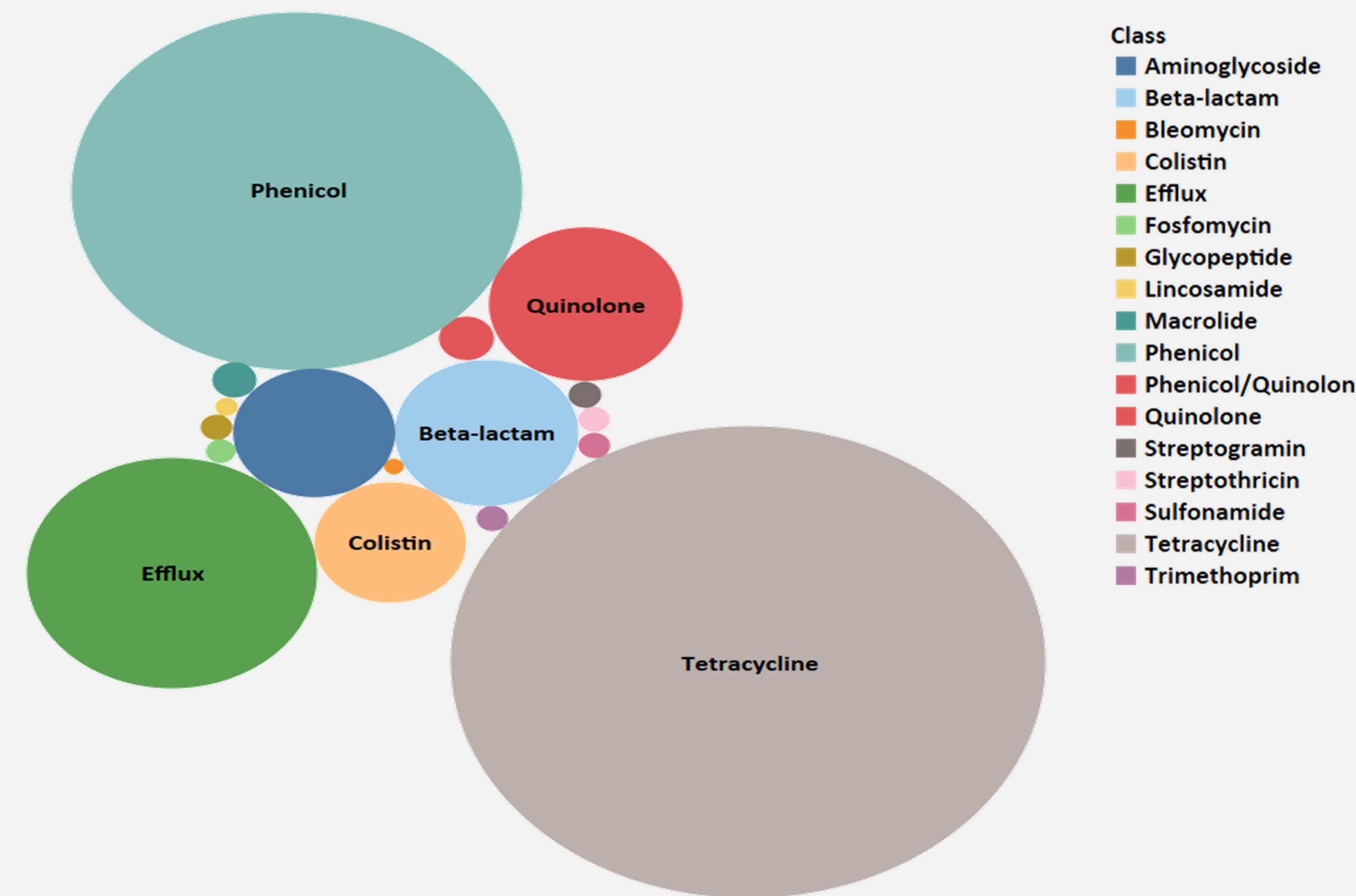


Figure 3. Bar graph showing antimicrobial resistance genes identified by class and mechanism. RPKM – reads per kilobase of the reference gene per million reads

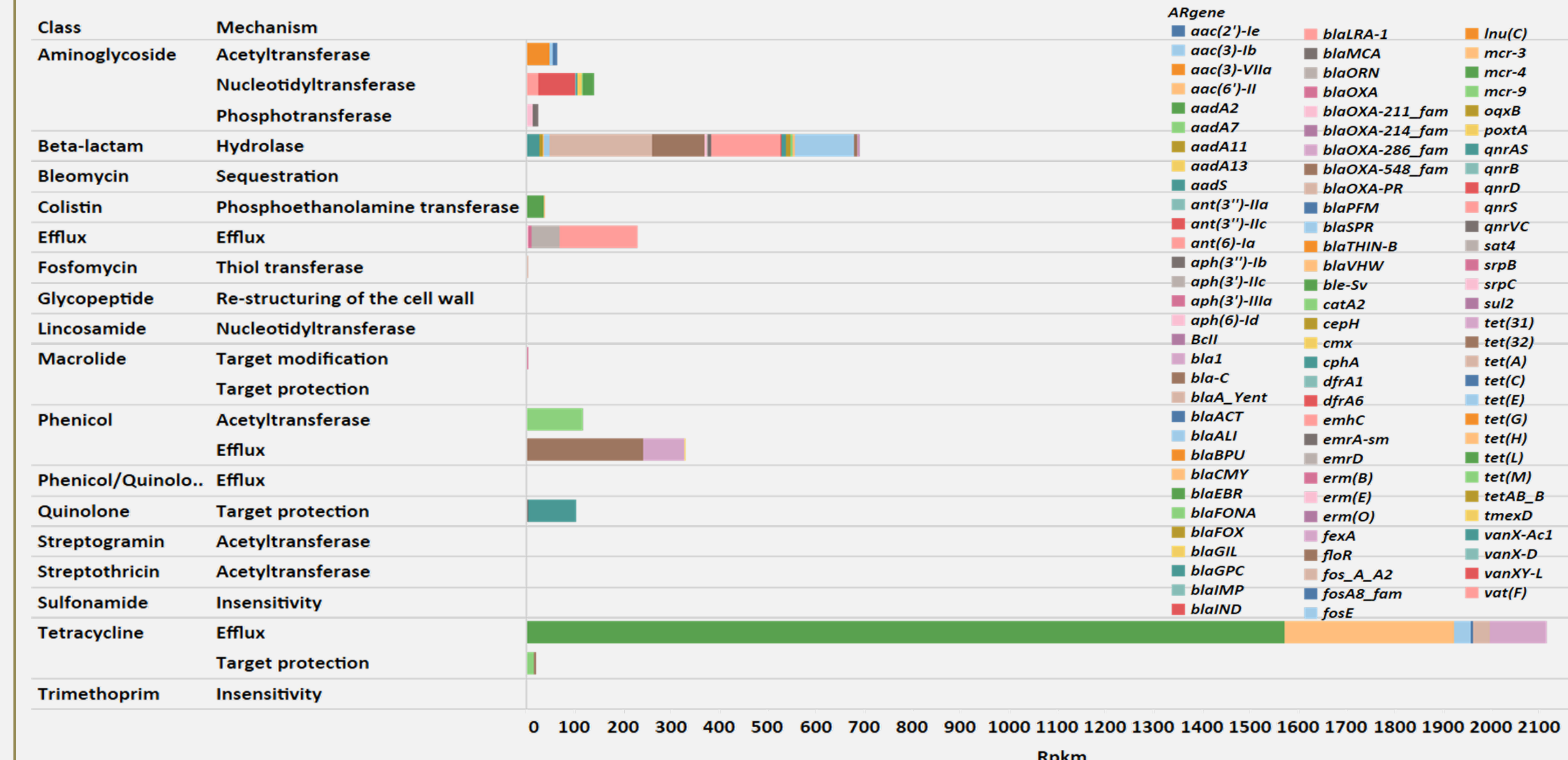


Figure 4. Bar graph showing antimicrobial resistance class identified by sample type

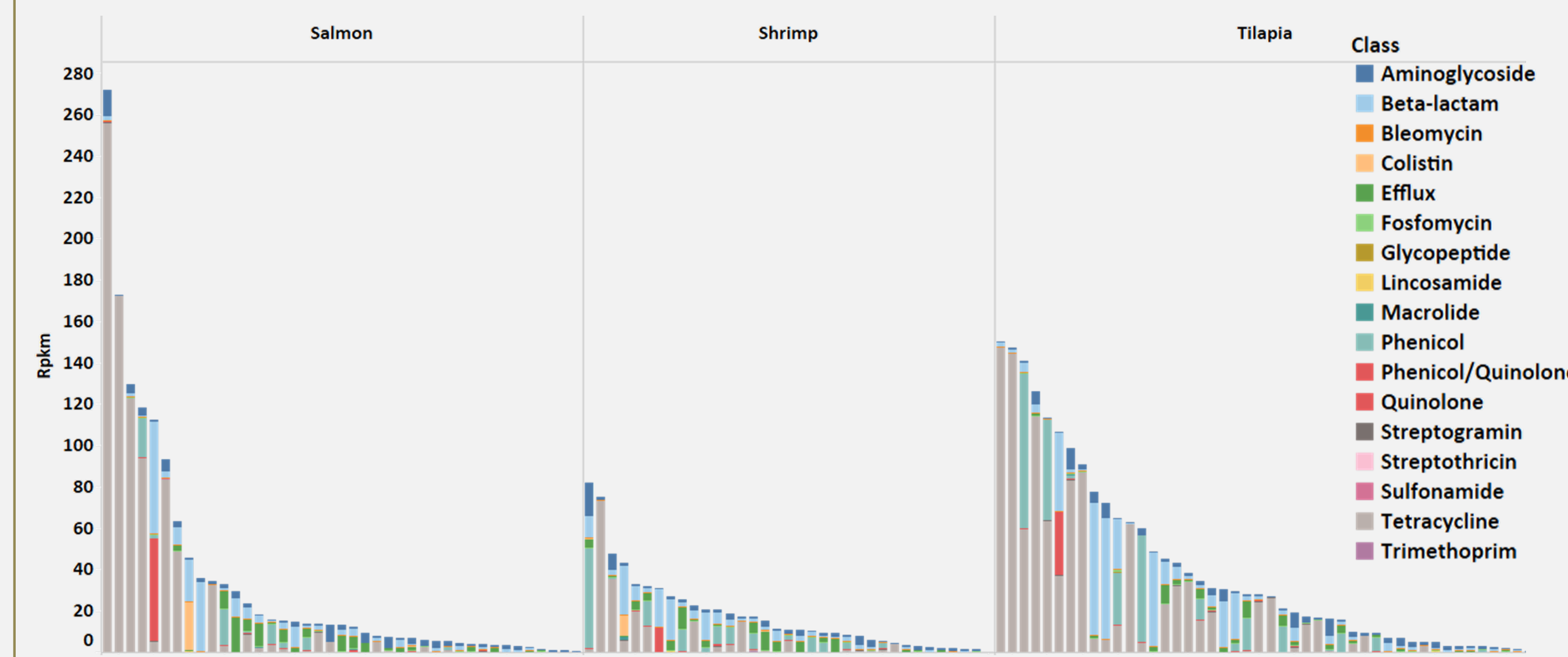


Figure 5. Bar graph showing the major antimicrobial resistance genes by sample types

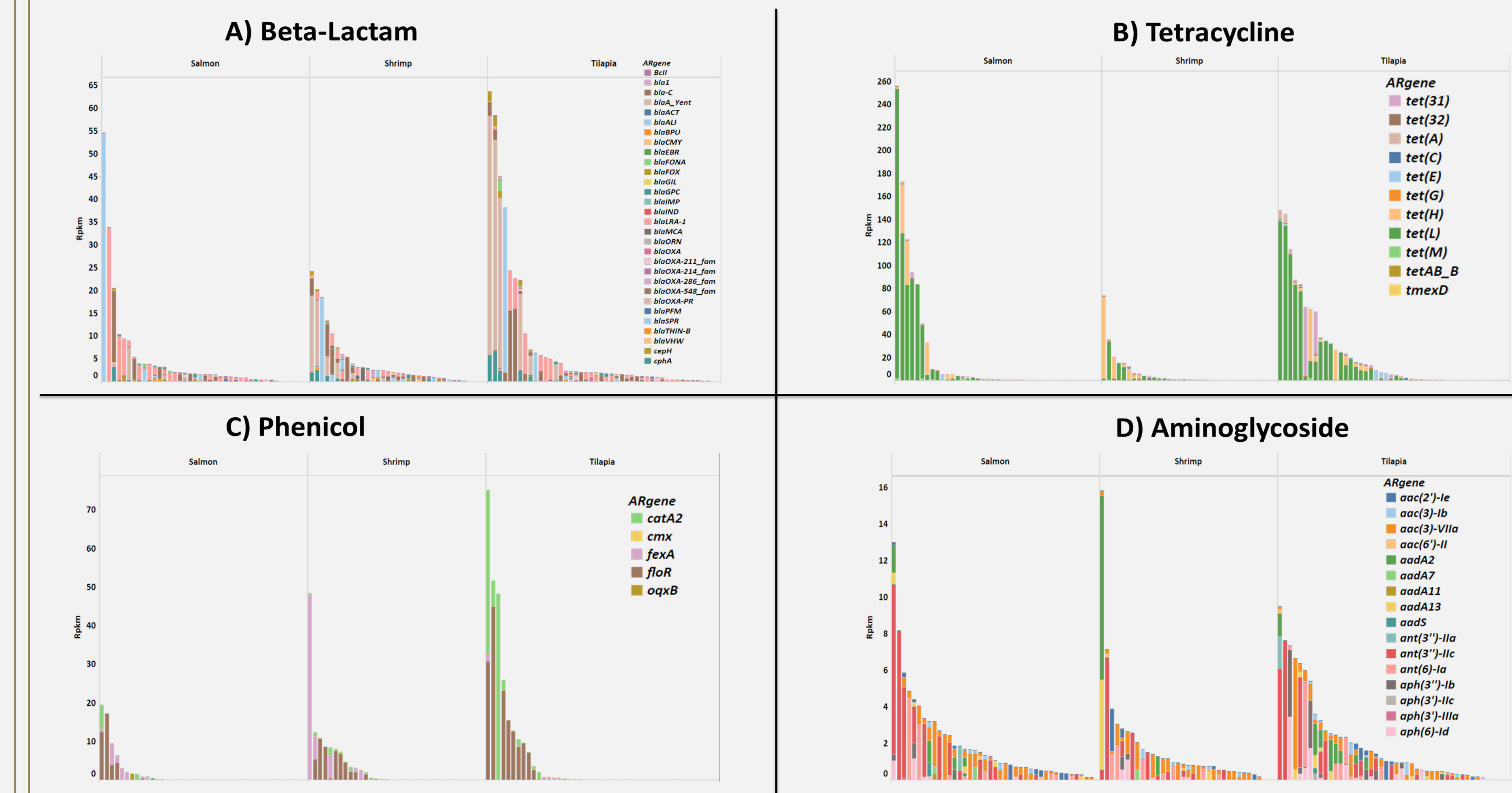


Figure 6. Network analysis of seafood resistomes

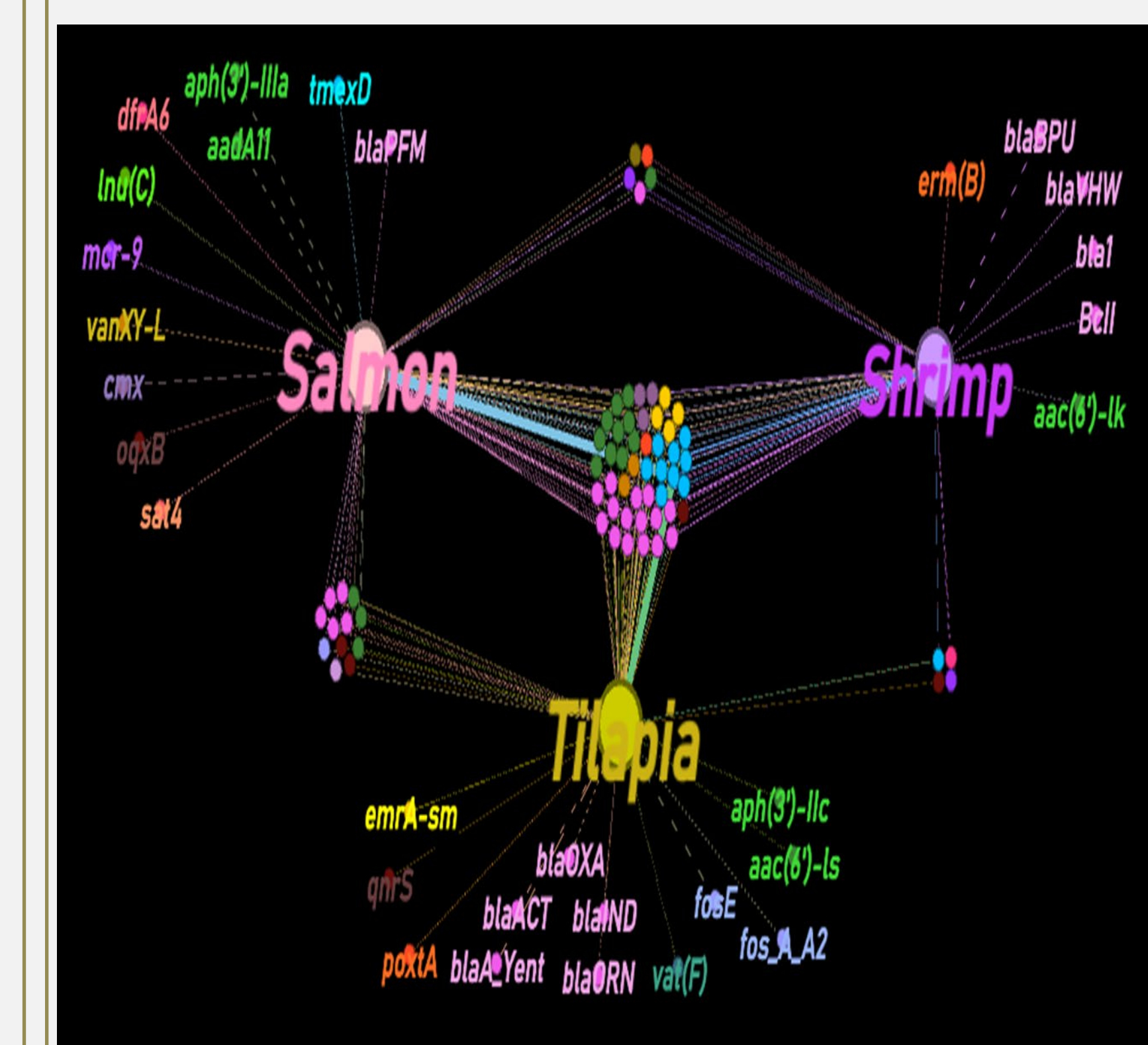
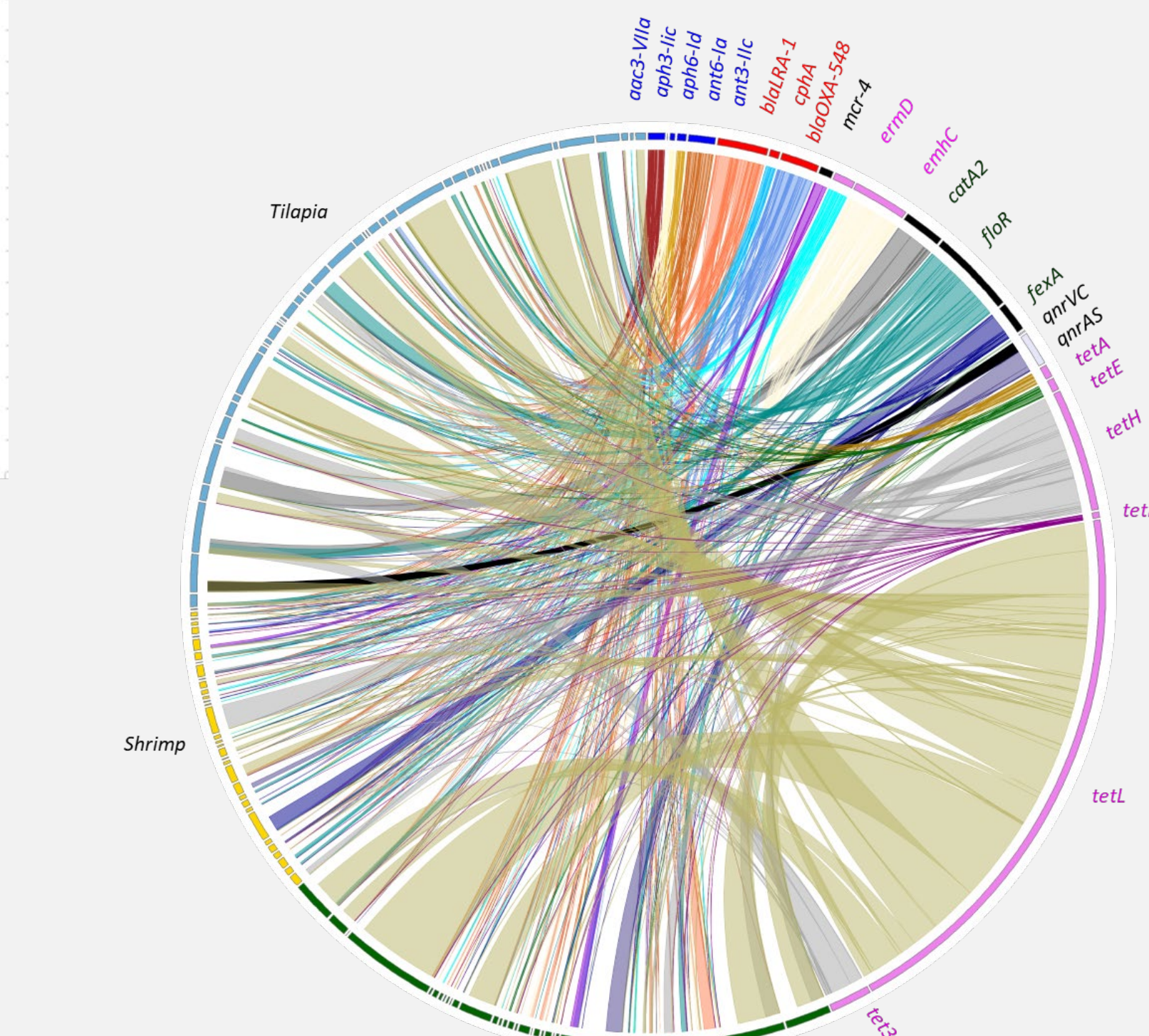


Figure 7. Circos representation of major ARGs among seafood samples



## Conclusions

- The resistome analyses identified more than 91 resistance genes representing 15 drug classes including more than 31  $\beta$ -Lactam resistance variants (including *bla<sub>OXA</sub>*, *bla<sub>IMP</sub>*, *bla<sub>CMY</sub>* and *bla<sub>FOX</sub>* genes) and quinolone (*qnrA*, *qnrB*, *qnrD*, *qnrS*, *qnrVC* and *oqxB*) resistance genes.
- The distribution and relative abundance of antibiotic resistance genes observed varied by seafood type.
  - The most common antimicrobial resistance genes observed:
    - Salmon - *aac(3)-VIIa* (70.7%), *bla<sub>LRA-1</sub>* (53.7%), *emhC* (46.3%) and *tet(L)* (46.3%)
    - Shrimp - *aac(3)-VIIa* (68.6%), *emhC* (51.4%) and *bla<sub>OXA-548</sub>* family (48.6%)
    - Tilapia - *aac(3)-VIIa* (58.7%), *bla<sub>OXA-548</sub>* family (58.7%) and *tet(L)* (56.5%)
- Our study provides insights into seafood resistomes and helps to establish baseline resistome levels to inform and prioritize public health management decisions aimed at identifying and reducing the source and exposure of resistance genes.

## Disclaimer

The views expressed in this poster are those of the author(s) and may not reflect the official policy of the Department of Health and Human Services, the U.S. Food and Drug Administration, or the U.S. Government.

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