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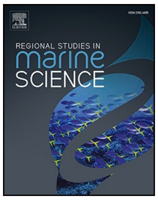

The impact of mariculture on the spread of multidrug-resistant bacteria and genes in South China's coastal waters; Insights from ecological conservation and coastal public health management strategies

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ABSTRACT

The purpose of this research was to determine whether mariculture settings in China harbor antibiotic-resistant bacteria, genes, or mobile genetic elements (MGEs). In mariculture waters spanning about 5000 km of South China's coastline, we examined the diversity, abundance, and co-occurrence of pathogenome, resistome, and mobilome using metagenomic and 16S rRNA studies. 207 pathogenic species were found; only 10 could be cultured; the rest were unculturable and were more common in mariculture waters than in nearby coastal regions. These results suggest that mariculture systems may facilitate the presence and potential spread of unculturable infectious diseases. Multidrug resistance genes predominated among the 913 subtypes we found across 21 ARG categories. The abundance of mobile genetic elements (MGEs), including plasmids, integrons, transposons, and insertion sequences, suggests that horizontal gene transfer is relatively standard. Pathogens, ARGs, and MGEs were strongly associated in co-occurrence network analysis, suggesting that many pathogens in mariculture settings may be multidrug-resistant. The interplay and interconnection of these factors highlight the potential influence of China's aquaculture practices on microbial communities and the spread of Resistance. To protect marine and public health against the development and spread of new antibiotic-resistant diseases, this research emphasizes the critical need for stronger antibiotic control in mariculture and improved monitoring.

1. Introduction

The rapid global expansion of aquaculture has prompted serious concerns about the emergence and spread of antibiotic resistance, driven by the extensive use of antibiotics to prevent and control aquatic diseases. About 57.5% of the world's aquaculture production comes from China, the industry leader (Wang et al., 2025). The widespread use of antibiotics in aquaculture contributes significantly to environmental concerns surrounding antibiotic resistance. This research set out to examine the pathogenome, antibiotic resistance genes (ARGs), and mobile genetic elements (MGEs) in mariculture waters spanning approximately 5000 kilometers of the South Chinese coastline to understand their distribution and interactions (Zhou et al., 2024). His study aimed to: (1) characterize the diversity and spatial distribution of the resistome, mobilome, and pathogenome, (2) investigate factors contributing to unculturable pathogens, and (3) evaluate their virulence potential. To thoroughly investigate the antibiotic resistance risk factors and co-occurrence patterns in different aquaculture environments, this work used high-throughput metagenomic sequencing in conjunction with 16S rRNA gene analysis (Zhao et al., 2019).

The ongoing existence and potential for antibiotic resistance genes to spread across many ecosystems have garnered increased interest, as they are seen as emergent environmental pollutants. Surface waters and underlying sediments in mariculture habitats in China have been shown to contain a broad diversity of ARGs and residual antibiotics (Gul et al., 2024). Many antibiotics, including tetracyclines, aminoglycosides, sulfonamides, β-lactams, and even colistin, which are last-resort medications, have been widely used without proper regulation, a practice linked to the prevalence of these resistance genes (Yan et al., 2022). According to research conducted in several mariculture zones, all sample sites evaluated showed the presence of numerous ARGs. Specifically, sulfonamide resistance genes (e.g., sul1, sul2, sulA) and tetracycline resistance genes (e.g., tetC, tetX) were frequently detected. In addition, the ARG concentrations in sediment samples were consistently higher

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than in water samples, indicating that sediments used in mariculture serve as permanent reservoirs for resistance genes. Multidrug resistance genes pose a significant threat to public health and food safety because they enable harmful bacteria to survive multiple antibiotic treatments (Anderson et al., 2025).

In ARG survival and dissemination within bacterial populations, mobile genetic elements are crucial. The ability to quickly acquire and disseminate resistance characteristics across phylogenetically distant microbial species is enabled by horizontal gene transfer (HGT), which is facilitated by mobile genetic elements (MGEs) such as plasmids, transposons, integrons, insertion sequences, and bacteriophages. MGEs are particularly abundant in the mariculture environment, which is subject to heavy human interference through practices such as intensive farming, antibiotic inputs, and nutrient loading (Mithuna et al., 2024). The development of antibiotic-resistant bacteria is accelerated by increased ARG mobilization, driven by the greater prevalence of MGEs. Consequently, it becomes much more challenging to manage and treat bacterial illnesses in both aquaculture and human populations when vulnerable bacteria in the mariculture environment develop Resistance via HGT. Network analysis in the research showed that ARGs and MGEs are highly correlated, lending credence to the hypothesis that mariculture waters are prime locations for genetic adaptation and exchange. Within this framework, MGEs serve as vectors of Resistance while simultaneously amplifying environmental and health hazards (Ahmad et al., 2022).

Thirdly, the pathogenome, or community of pathogenic bacteria in the mariculture waters (containing strains that can be cultured and those that cannot), is studied in this work (Nasir et al., 2023). Having these genes carried by harmful species makes the pathogenicity potential of ARGs even more worrisome. Of the 207 pathogen species found, only 10 could be cultured in a typical lab setting. Unculturable infections predominate in these habitats, yet regular monitoring fails to detect them. The virulence genes of these pathogens can be preserved even when they are in a viable but non-culturable (VBNC) state, allowing them to regain pathogenicity when revived in an ideal environment or with a suitable host. Pathogens detected include *Vibrio cholerae*, Acinetobacter baumannii, Pseudomonas aeruginosa, and Klebsiella pneumoniae (Jin and Jin, 2021). Among these, several species are multidrug-resistant and are considered of high importance. As an example, cholera and gastroenteritis may develop during resuscitation if certain Vibrio strains cannot be cultured. In a similar vein, A. baumannii in a VBNC state was discovered to upregulate adhesion genes linked to virulence when exposed to high-salinity environments. In addition to surviving, these uncultivable pathogens may act as ARG carriers and transfer agents in the microbiome, highlighting the underappreciated danger they pose (Anh Khoa et al., 2020).

Our knowledge of environmental antibiotic resistance in coastal aquaculture environments is greatly enhanced by this work, which provides several significant contributions. We provide a comprehensive picture of the causes of antibiotic resistance in mariculture waters in South China by combining data from the mobilome, pathogenome, and antibiotic resistome. Horizontal gene transfer is a key factor in the spread of Resistance, as evidenced by the high ARG diversity, the prevalence of multidrug resistance subtypes, and the significant association with MGEs. Another environmental health risk that standard monitoring methods may miss is the presence of virulent, multidrug-resistant, unculturable microorganisms. These results emphasize the critical need for improved surveillance systems that incorporate molecular detection techniques capable of capturing VBNC and culturable infections. In addition, policymakers and risk assessors working on antibiotic usage in aquaculture may benefit from this study's findings. To reduce the likelihood of antibiotic resistance in these ecosystems, it is essential to implement stronger restrictions on antibiotic usage, enhance water quality management, and encourage the use of non-antibiotic methods of disease control. The findings of this research have important implications for international initiatives to curb the spread of antibiotic-resistant bacteria and for the long-term viability and security of aquaculture as a whole.

2. Materials and methods

2.1. Collecting samples and measuring water quality

We collected 21 surface water samples from seven representative mariculture zones along about 5000 kilometers of coastline in South China between April and June 2021 to study the microbial community structure, antibiotic resistome, mobilome, and pathogenome in mariculture waters. These mariculture locations were chosen: Shuimentan (SMT), Sanniangwan (SNW), Yingzi (YZ), Nanshandao (NSD), Yangjiang (YJ), Zhuhai (ZH), and Huizhou (HZ). For comparison, two control sites, Paotai (PT) and Dongaodao (DAD), were also included and had no mariculture activities nearby. Guangxi (SMT, SNW, PT) and Guangdong (YZ, NSD, YJ, ZH, HZ, DAD) were the two central coastal provinces where the mariculture locations were located. The supplemental materials provide the exact geographic coordinates and information of the sample location (Sampantamit et al., 2020).

We took samples from three separate ponds at each mariculture site at random. Four different locations, each 50 cm below the water's surface, were randomly chosen to collect water samples from each pond. Each location gathered 2 liters of water in total (Cerca et al., 2024). To ensure that the samples remained unaltered by microbes throughout transportation, they were promptly placed in sterile containers, placed on ice, and brought to the lab in the dark.

A multiparameter water quality analyser was used to detect critical environmental factors such as pH, salinity, temperature, and dissolved oxygen in real-time. The dichromate oxidation technique was used to assess chemical oxygen demand, and ammonium molybdate colorimetry was used for spectrophotometric analysis of total phosphorus. These indicators of water quality were documented at each location and are available in the supplemental data tables. Three biological replicates were combined from each location before DNA extraction and microbiological analysis to ensure representativeness. Isolation of bacterial colonies from cultures was also facilitated by this composite sampling method (Castillo et al., 2023).

DNA was extracted from each filtered water sample using the *DNeasy PowerWater Kit* (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The same kit was used for both metagenomic and 16S rRNA sequencing to ensure consistency. DNA concentration and purity were assessed with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and quantified using a Qubit 3.0 fluorometer (Invitrogen, USA).

2.2. Sequencing of the metagenome

Nextera XT DNA Library Prep Kits were used to prepare metagenomic DNA libraries according to the manufacturer's instructions (Illumina, San Diego, CA, USA). The Illumina NovaSeq 6000 platform with a paired-end read length of 2 × 150 bp was used for sequencing, producing approximately 1012 Gbp of raw data per sample. Raw reads were initially evaluated with FastQC v0.11.9 for quality, and Trimmomatic v0.39 was used to trim them to remove low-quality bases and adapter sequences. Host-derived reads were filtered and mapped to the reference genome with Bowtie2 v2.5.1. The de novo assemblies were done using MEGAHIT v1.2.9, and Prokka v1.14.6 was used to predict open reading frames (ORFs). ARGs were identified by matching against CARD (Comprehensive Antibiotic Resistance Database, v2023) and SARG using BLASTp with$\geq$ 90% identity and $\geq$ 70% coverage. ISfinder, INTEGRALL, and PlasmidFinder were used to annotate mobile genetic elements (MGEs) (Fu et al., 2021; Delgado et al., 2021).

2.3. 16S rRNA gene amplicon sequencing of bacteria that can be cultivated

The V3–V4 region of the bacterial 16S rRNA gene was amplified using primers 341 F (5′-CCTACGGGNGGCWGCAG-3′) and 805 R (5′-GACTACHVGGGTATCTAATCC-3′). Amplicons were sequenced on the Illumina MiSeq platform with paired-end reads of 2 × 300 bp. On average, each sample produced 50,000–70,000 raw reads. Quality filtering was performed using QIIME2 v2023.2, which included demultiplexing, chimera removal with DADA2, and denoising to obtain amplicon sequence variants (ASVs). Taxonomic assignment was carried out using the SILVA v138 database with a confidence threshold of 97% (Li et al., 2021; Zhang et al., 2023).

2.4. Bioinformatics and annotation

Metagenomic clean reads were assembled using MEGAHIT v1.2.9, and open reading frames (ORFs) were predicted with Prokka v1.14.6. Annotation of antibiotic resistance genes (ARGs) was performed using BLASTp against the CARD (Comprehensive Antibiotic Resistance Database, v2023) and SARG databases ($\geq$90% identity, $\geq$70% coverage). Mobile genetic elements (MGEs) were identified using ISfinder, INTEGRALL, and PlasmidFinder. Virulence factors were annotated using the VFDB (Virulence Factors of Pathogenic Bacteria, v2023). For 16S rRNA gene sequences, taxonomic classification was conducted with QIIME2 v2023.2 against the SILVA v138 reference database at a 97% similarity threshold.

2.5. Analysis

The antibiotic resistome, mobilome, and pathogenome were analyzed using a variety of visualization and statistical methods to determine community structures and patterns of co-occurrence. Networks of co-occurrence were built using relative abundance data and Pearson's correlation coefficients. The network analysis only kept statistically significant positive correlations ($r > 0.5$ and $P < 0.01$). Gephi was used to visualize networks, enabling investigation of the intricate interplay among ARGs, MGEs, and pathogens (Liu et al., 2022). Abundance profiles of pathogenic and resistant components across various mariculture and control locations were compared using a heatmap. Because of this, hotspots could be more easily located, and the relative importance of each site to the overall resister and pathogenome burden could be better assessed. To better understand community overlap and site-specific resistance patterns, we used Venn diagrams to show the amount of common and unique ARG subtypes across sample sites (Kumar et al., 2023).

Bray-Curtis dissimilarity measurements were used in principal component analysis (PCA) to uncover larger trends in microbial composition. When comparing pathogen community structure and antibiotic resistance gene spectrum across different sample sites, principal component analysis was functional (Dao et al., 2019). Ecological statistics software was used to perform these multivariate analyses. Taken as a whole, these methods enabled a thorough evaluation of the dynamics of mariculture microbial communities and the factors that promote the spread of Resistance.

3. Results and discussion

3.1. Mariculture waters as traps for pathogens, antibiotic resistance, and mobile organisms

3.1.1. Pathogenome diversity and its potential

Metagenomic sequencing, culturable evaluations, and 16S rRNA gene analysis were used to classify pathogen populations in mariculture waters. Bacteria dominated all samples at the community kingdom level, accounting for 83.8–99.2% of the total microbial communities. One sample had the highest bacterial percentage (99.2%) among all locations. The most prevalent microbial groups across all samples were Proteobacteria, Bacteroidetes, and Actinobacteria, among 237 phyla and more than 6000 species. Mariculture waters had much lower levels of Proteobacteria than control locations, suggesting that this microbe is more common in clean, undisturbed saltwater (Dyrset, 2025).

Metagenomic profiling revealed the presence of 207 pathogenic species across all samples, of which bacteria constituted the dominant fraction (77.0–82.6%) (Fig. 1a). In total, 181 pathogenic bacterial species were detected in both mariculture and control waters, with highly similar community compositions observed between Guangdong and Guangxi mariculture sites and notable overlap with adjacent control locations, indicating possible pathogen dispersal from aquaculture activities (Fig. 1b–c). Multidrug resistance genes accounted for approximately 35% of the total ARG abundance and were significantly enriched in aviculture waters compared with controls (Krystal–Wallis test, $\chi^2 = 14.27$, $p = 0.003$). The most prevalent pathogens included *Staphylococcus aureus*, *Salmonella enterica*, and *Pseudomonas aeruginosa*, which are associated with gastrointestinal, respiratory, and skin infections through exposure to contaminated water or seafood (Fig. 1e) (Maulu et al., 2021). A significant abundance of pathogenic organisms was observed at the control site compared with nearby mariculture sites, suggesting environmental spillover of disease from aquaculture zones. Pathogen communities in mariculture samples from Guangdong and Guangxi clustered closely within the same confidence ellipse in a non-metric multidimensional scaling (NMDS) analysis. In contrast, control samples were positioned separately, suggesting compositional differences from waters affected by mariculture (Tom et al., 2021). As a result, we can say with confidence that mariculture waters, unlike the mostly unspoiled control zones, host a diverse and abundant pathogenome.

Overall, mariculture waters along the South China coast harbor diverse pathogenic bacteria, including both culturable and unculturable forms, with evidence of microbial contamination extending to adjacent coastal waters. The presence of clinically relevant pathogens such as *Salmonella enterica*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio cholerae*, *Mycobacterium tuberculosis*, and pathogenic *Escherichia coli* highlights the need for strengthened management strategies and enhanced monitoring to reduce pathogen dissemination and protect marine ecosystem health.

Culturable bacterial abundances in mariculture waters ranged from 10^5 to 10^6 CFU mL^{-1} , exceeding control levels by one to two orders of magnitude. Analysis of 16S rRNA sequences identified 234 bacterial genera across 14 phyla, with Proteobacteria and Bacteroidota predominating and *Vibrio* and *Pseudoalteromonas* as the most abundant genera. Alpha diversity indices indicated significantly higher microbial diversity in mariculture waters, a pattern confirmed by a Kruskal–Wallis test ($\chi^2 = 11.62$, $p = 0.003$) (Chen et al., 2023a). The dominance of Proteobacteria suggests that nutrient availability plays a vital role in shaping microbial community structure, consistent with observations from other mariculture environments. Using a curated database of human pathogens, 15 culturable pathogenic species were identified, with 9 commonly detected in mariculture waters and 3 present across all samples. *Vibrio cholerae* and *Bartonella henselae* were the most prevalent culturable pathogens, while *Salmonella enterica* and *Pseudomonas aeruginosa* showed high relative abundances in several mariculture sites, exceeding 8% in individual samples (Conroy et al., 2022). Comparison of metagenomic and culture-based approaches showed that only a small subset of pathogenic species was detectable by cultivation. While two species were detected in all samples and both methods identified 10, metagenomic analysis alone identified 196 pathogenic species, indicating a predominance of unculturable pathogens. These findings suggest that mariculture waters support dense and diverse pathogenic communities compared with nearby control sites (Chen et al., 2023b). Although control sites contained lower absolute levels of pathogenic bacteria, the relative abundance of dominant species was comparable to that in

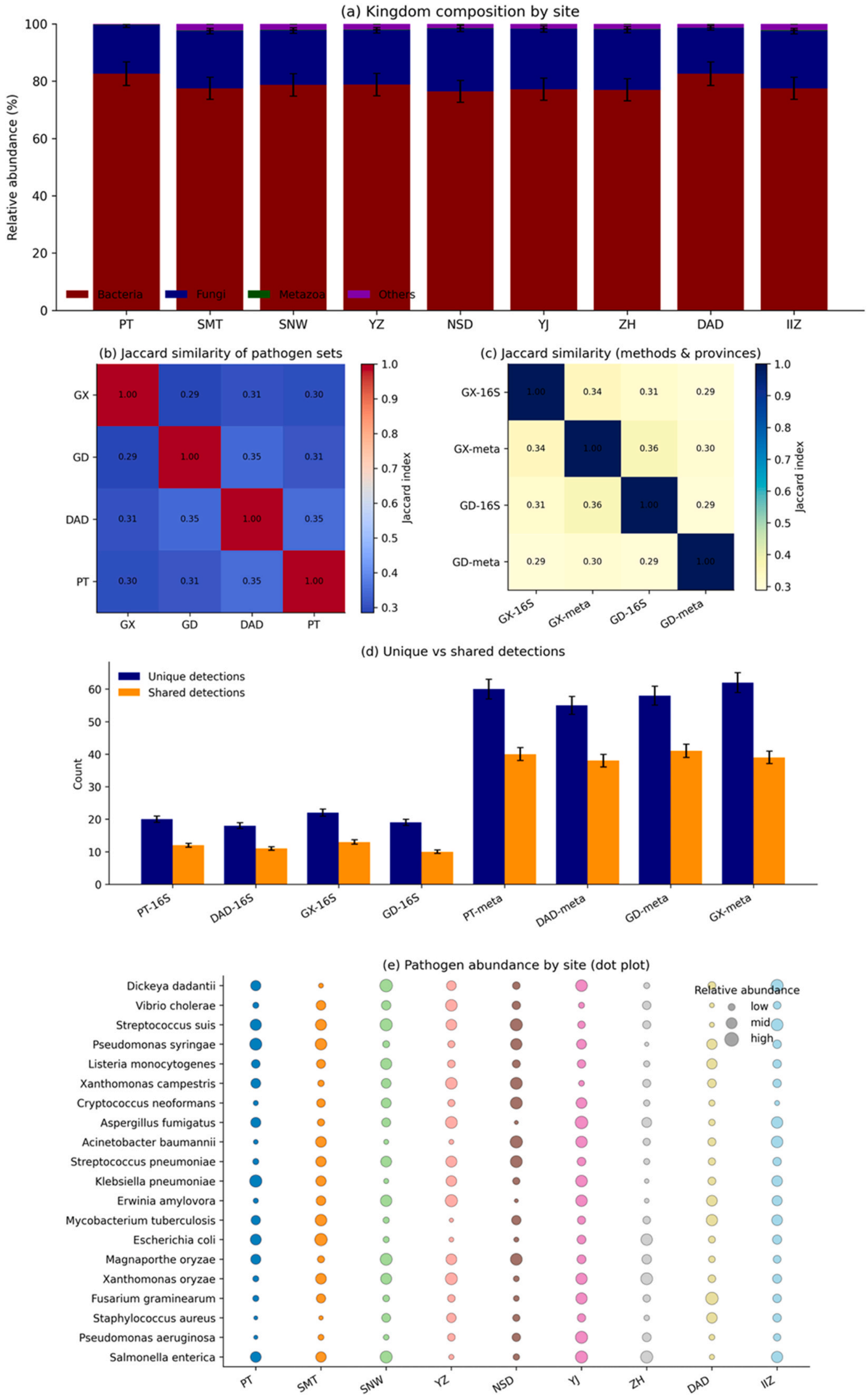


Fig. 1. shows the distribution and levels of harmful microorganisms in the mariculture and control water samples. In (a), phylum-level percentages are shown; in (b) and (c), pathogenic species shared across mariculture and control locations are shown using metagenomic and 16S rRNA data; in (d), culturable and metagenomic pathogens are compared; and in (e), a heatmap of species-level abundance is shown. Sites PT and DAD serve as controls, whereas GD and GX stand for Guangdong and Guangxi, respectively.

mariculture waters, suggesting possible pathogen spread into adjacent coastal ecosystems. This dissemination may result from aquaculture-related inputs such as untreated effluents, direct water discharge, and contaminated feed, which together can facilitate the continuous circulation of pathogenic bacteria in marine environments (Sathish et al., 2023). The predominance of pathogens detected exclusively by metagenomic sequencing indicates that many bacteria in mariculture waters exist in unculturable yet potentially virulent states, posing hidden risks to food safety and environmental health. Environmental stressors such as salinity, pH, and solar exposure may induce viable but non-culturable (VBNC) conditions in pathogens, including *Staphylococcus aureus*, *Salmonella enterica*, and *Vibrio cholerae*. In this study, salinity ranged from $< 5‰$ to $\sim 38‰$, and more than 94% of detected pathogens were not recoverable by cultivation. Notably, *Pseudomonas aeruginosa* and *V. cholerae* were consistently detected across all samples, with *V. cholerae* showing high culturability despite low metagenomic abundance, suggesting its potential as an indicator organism in mariculture environments (Sierra-Correa et al., 2024). Analysis of environmental conditions showed that specific pathogens, such as *Salmonella enterica*, were associated with low-salinity waters and were absent from high-salinity sites, likely due to stress-induced transitions to unculturable states. For example, *S. enterica* was abundant at sites with salinity below 5‰ but undetected at sites with salinity above 22‰, supporting a negative relationship between salinity and pathogen culturability. Among unculturable pathogens, *Staphylococcus aureus* was the most prevalent, underscoring its potential as a foodborne pathogen. Together, metagenomic, culture-based, and 16S rRNA analyses demonstrate that mariculture waters along the South China coast harbor diverse pathogens with varying culturability, which may readily disperse from aquaculture zones into adjacent coastal ecosystems (Jayathilaka et al., 2023). The widespread presence of unculturable yet potentially infectious pathogens poses a significant challenge for environmental monitoring and food safety, underscoring the need for improved molecular detection approaches and stricter regulatory oversight in aquaculture systems.

3.1.2. A community of microbes with a wide range of antibiotic resistance

Analyzing the antibiotic resistome profiles in mariculture waters allowed researchers to assess the impact of mariculture on the coastal marine environment. Fig. 2 summarizes the antibiotic resistance gene (ARG) diversity, abundance, and mechanisms across all water samples. Nine hundred and thirteen ARG subtypes were identified, representing twenty-one different resistance groups. These groups mostly matched the antibiotic classes most commonly used in humans, such as peptides, tetracyclines, macrolides, and streptogramins (MLS). In every sample, multidrug resistance genes accounted for about 35% of the overall ARG abundance, making them the most abundant ARGs (Fig. 2a). Resistance to tetracyclines and MLS ensued. It is worth noting that the control locations had far lower ARG subtype abundance and richness than mariculture waters. The resistome hotspot potential was highlighted by the SNW sample, which had the most extraordinary richness of ARG subtypes. On the other hand, the control samples showed lower overall abundance and fewer ARG species, indicating that those locations were under less antibiotic pressure (Sivadas et al., 2021).

Fig. 2b shows that, among the MLS genes, macB was the most prevalent, followed by oleC. This was based on further study of resistance types. Fig. 2c shows that a greater variety of ARG subtypes was associated with multidrug Resistance than with other categories. In contrast, the number of multidrug resistance genes was essentially constant across all samples. The high abundance of genes such as msbA and evgS across almost all samples indicates that these genes are commonly found in mariculture settings.

Although the number and variety of tetracycline resistance genes were decreased in the control waters, they were nonetheless extensively dispersed (Fig. 2d). Notably, compared to mariculture locations, the levels of tetracycline-related ARG were lowest in the ZH sample. These results indicate that ARG accumulation is driven by continual antibiotic input from aquaculture, particularly multidrug-resistant and MLS ARGs. Overuse of antibiotics promotes the persistence and spread of ARGs, as shown by the much greater ARG enrichment in mariculture waters compared to controls. This raises concerns about the spread of ARGs into surrounding ecosystems, which might endanger human and environmental health.

Antibiotic efflux accounted for around half to sixty percent of the overall resistance activity, making it the most prominent resistance mechanism seen in all mariculture water samples. Afterwards, strategies like target protection, target replacement, and target change emerged. A total of 40 ARGs were identified; among them, multidrug resistance genes confer Resistance to 17 different classes of antibiotics, including nitroimidazoles, fluoroquinolones, MLS, and others. The results indicate that mariculture waters tend to acquire a variety of genes that make it harder to treat diseases caused by bacteria resistant to many drugs. This poses a serious concern (Ren et al., 2024).

In this sample, we identified a large number of genes conferring Resistance to multiple antibiotics, including macB, oleC, and optrA. For instance, the rpoB2 gene may change its target or substitute itself to function. Another member of the ABC superfamily that is associated with multidrug Resistance is msbA, a transporter protein homolog. Bacteria can quickly become resistant to antibiotics thanks to these transporter proteins, which serve as their primary defence. Efflux pumps should be closely monitored since they play a significant role in bacterial Resistance, whether the bacteria are Gram-negative or Gram-positive.

Antibiotic resistance genes (ARGs) originating in mariculture regions may have migrated to neighbouring coastal ecosystems, as the control sites PT and DAD showed ARG profiles comparable to those in mariculture waters. Fig. 3 shows that redundancy analysis (RDA) confirmed this trend, indicating that ARGs such as evgS and macB were prevalent in both the DAD and YZ samples. The majority of ARGs were found near samples YZ and NSD, which also exhibited robust relationships with environmental variables. The RDA revealed a positive association between salinity and pH and significant ARGs, including evgS, msbA, and macB, and a negative association with total phosphorus. The first RDA axis accounted for 21.9% of the overall variance, whereas the second axis explained 14.2%. The data suggest that higher salt levels in mariculture waters increase ARG concentrations, possibly through increased bacterial stress responses, elevated ARG gene expression, or horizontal gene transfer.

On the other hand, ARG persistence or expression may be diminished by elevated phosphorus levels. The concentration and variety of ARGs linked to glycopeptides, peptides, aminocoumarins, mupirocin, and β-lactams were notably higher in mariculture waters than in control locations. Mariculture sites in Guangdong and Guangxi, including SNW, YZ, NSD, and YJ, require close monitoring due to elevated ARG levels. It is worth noting that the levels of ARGs in mariculture samples from the nearby sites, ZH and HZ, were lower than those in the control site DAD. This might mean that ARGs are being carried or deposited in the nearshore seawaters by runoff or tides (Wan et al., 2024).

There are serious environmental concerns due to the abundance and variety of ARGs resistant to many drugs in mariculture waters and nearby coastal areas. Among them are hazards to marine life, contamination of seafood, and potential exposure of human-associated microbes to ARGs in the environment or through food. To slow the spread of aquaculture-related resistance into other ecosystems, preventive measures and regulatory constraints are crucial.

In general, ARGs resistant to many drugs have been found in mariculture waters throughout the 5000 km coastline of southern China. Furthermore, a wide variety of complex ARGs appears to have been introduced into adjacent coastal waters through the discharge of untreated mariculture effluents. Proliferation of bacteria resistant to antibiotics is heightened in these conditions because the antibiotic resistome and pathogenome overlap spatially.

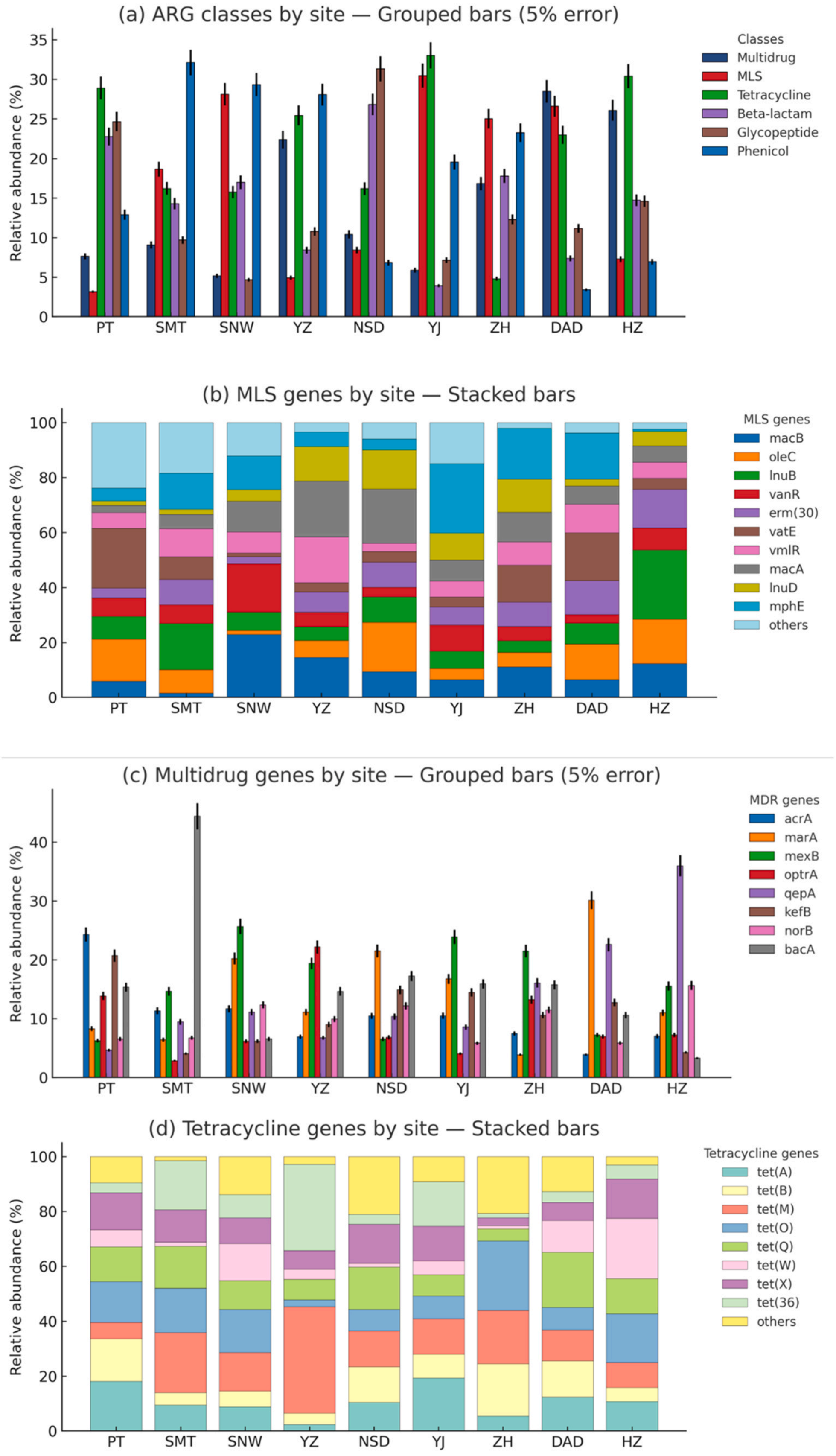


Fig. 2. Macrolide, multidrug, and tetracycline ARG relative abundances and top resistance subtypes across samples. Each resistance class's colour gradient represents the relative abundance of an ARG subtype.

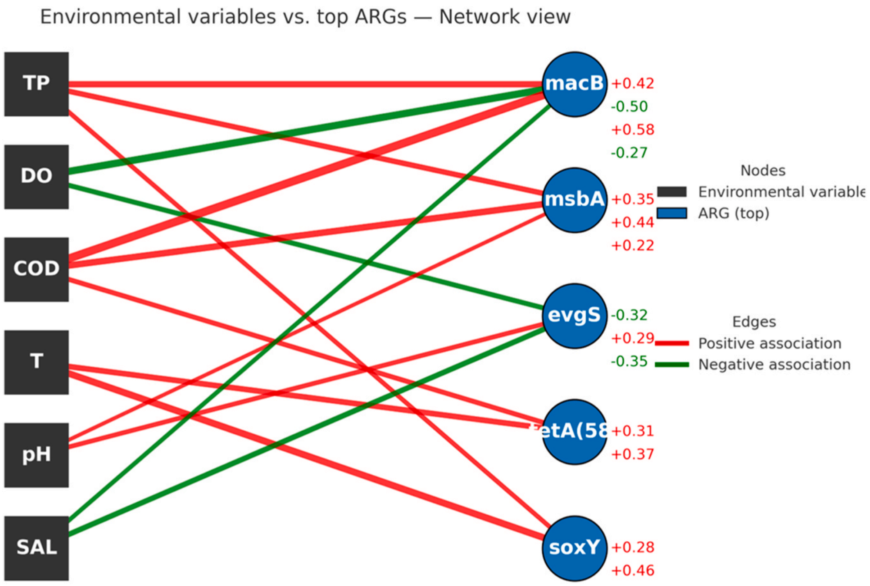


Fig. 3. Analysis of environmental variables' redundancy concerning the top 10 ARGs. TP stands for total phosphorus, AS for control sites, DO for dissolved oxygen, COD for chemical oxygen demand, T for temperature, and SAL for salinity.

3.1.3. Ample mobility making it easier to move ARGs from one mariculture water system to another

To determine the possibility of antibiotic resistance gene (ARG) horizontal gene transfer (HGT), BLAST was used to compare metagenomic data from all water samples with databases of integrons, transposons, insertion sequences, and plasmids. Strict criteria, such as an e-value $< 10^{-5}$ and an identity$>$ 90%, were imposed to ensure accurate identification. In all samples, the most diverse mobile genetic elements (MGEs) found were plasmids and insertion sequences (Fig. 4a). The following genetic elements were found: 7983 plasmids, 1801 insertion sequences, 72 transposons, and 8 integrons. These four MGE groups were subsequently investigated for gene type distribution and relative abundance (Fig. 4b). Plasmids were the most species-rich category; however, different plasmid types were not particularly abundant. Notably, compared to mariculture samples from Guangdong, the control sample DAD had a wider range of plasmid species (Balakrishnan Nair et al., 2024). On the other hand, SNW from Guangxi showed the most incredible total variety of plasmids among mariculture waters.

Many plasmids were shown to have originated from bacteria, as determined by taxonomic analysis (Fig. 4c), including Ralstonia solanacearum and Rhizobium leguminosarum. Notably, human infections such as Vibrio, Klebsiella pneumoniae, and Salmonella enterica were also linked to several plasmids. The PT sample contained a high concentration of plasmids derived from Vibrio, with 93.5% of them in a circular form. This indicates that the PT sample has a high potential for gene transfer and mobility. The rate of genetic exchange among microbial communities is often inversely proportional to the quantity of MGEs. Because they mediate conjugation and transformation, plasmids are essential for horizontal gene transfer (HGT). Hence, mariculture systems show great promise for plasmid-mediated ARG transfer, as evidenced by the high plasmid abundance. Mariculture waters are hubs for genetic exchange and the transmission of resistance traits, as shown by the large number and diversity of plasmids, particularly those associated with pathogenic species. Overall, the preponderance of plasmids and insertion sequences in mariculture conditions, together with the mobilome, lays the groundwork for the rapid spread of ARGs. To evaluate the ecological and public health concerns associated with mariculture practices, it is crucial to monitor MGEs and ARGs.

A total of three types of integrons were detected in the samples; intI1, intI, and intIA were the most common. Three types of integrons were discovered at the control sites DAD and PT. In contrast, only two were found in the YJ mariculture sample (Fig. 4d). The fact that integrons were only found in trace amounts among the MGEs indicates that they may not have had much of a role in horizontal gene transfer (HGT) in these seas.

Although their relative abundance was not the highest, sample ZH had the most extensive variety of transposons (Fig. 4e). As a counterpoint, sample SNW had the most incredible proportionate abundance of MGEs but the fewest transposon kinds. In every sample, retrotransposons accounted for 60.6% of the total, with DNA transposons accounting for the remaining 39.4%. In general, the small number of transposons suggests that their function in facilitating ARG transfer in mariculture is minimal. Fig. 4f shows that insertion sequences were much more common in mariculture samples than in controls. Most notably, ISPfe2 stood out among the others. Mariculture waters routinely had greater abundances of the top 10 insertion sequences, suggesting that insertion sequence-mediated HGT may be common (Bonamano et al., 2024). By conjugating conjugative plasmids to various bacterial hosts, these elements facilitate gene transfer, increasing the likelihood of ARG diffusion between phylogenetically distant species.

Although they were less numerous, integrons and transposons contributed to the genetic mobility landscape, especially compared with plasmids and insertion sequences, which showed the most incredible variety and abundance. The prevalence of MGEs in mariculture environments suggests that there are several paths for HGT (Hu et al., 2023). Finally, a robust mobilome, including transposons and integrons, as well as plasmids and insertion sequences, is present in mariculture waters along the 5000 km coast of southern China.

3.2. The presence of both antibiotic resistance genes and mobilome information points to mariculture waters as a potential breeding ground for novel antibiotic-resistant pathogens

These transposable DNA elements lay the groundwork for the horizontal transmission of ARGs, underscoring the importance of mariculture systems as hotspots for the evolution of antibiotic resistance in the ocean. Relationships among dominant ARGs, important pathogenic species, and major mobile genetic elements (MGEs) were examined to explain the transfer of antibiotic resistance genes (ARGs) among pathogenic bacteria in mariculture waters. Fig. 5a shows the results of the co-occurrence analysis, which showed that many different pathogenic microorganisms were strongly associated with multidrug resistance ARGs.

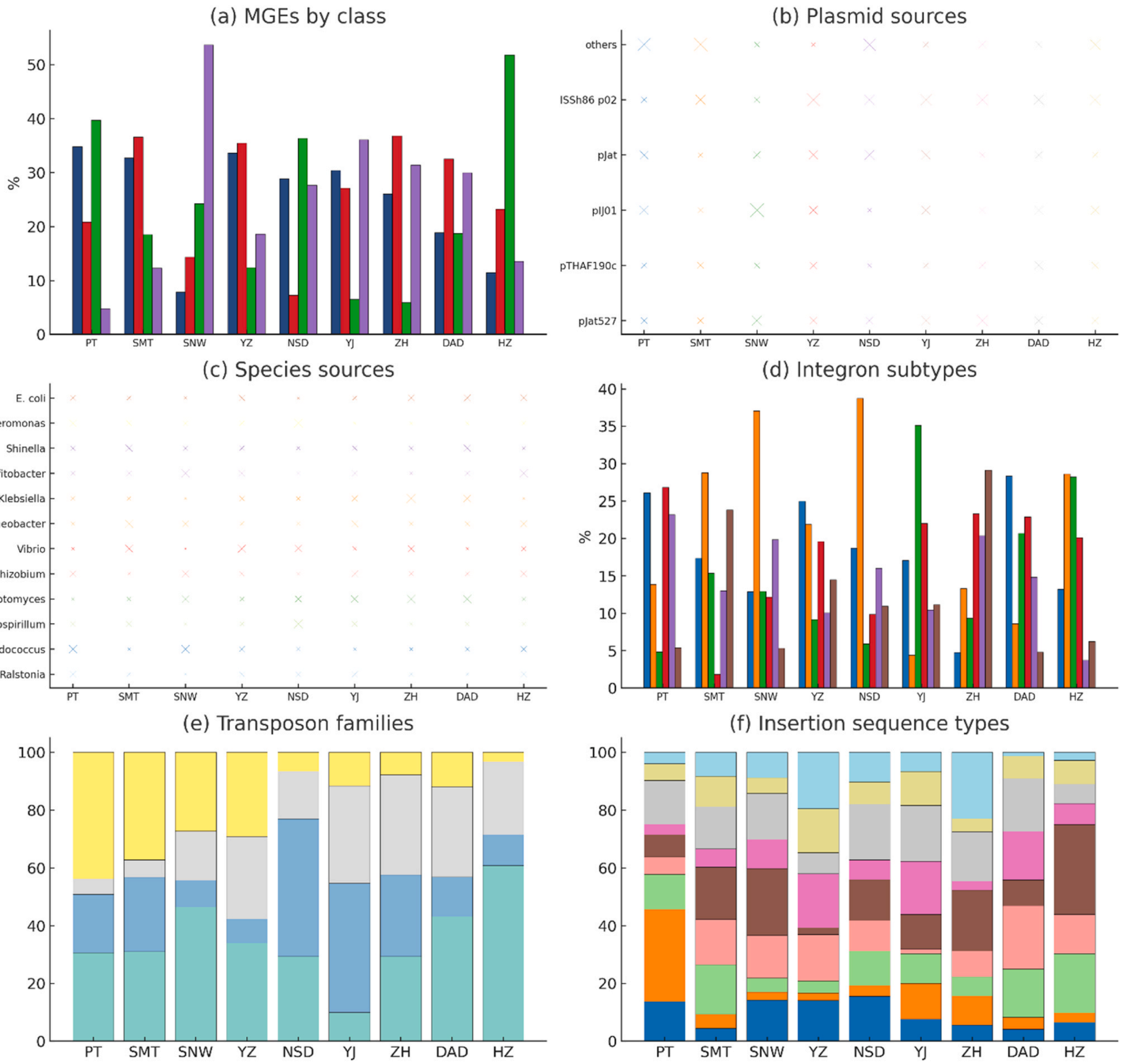


Fig. 4. (a) The proportion of mobile genetic elements (MGEs) such as plasmids, integrons, transposons, and insertion sequences; (b) the distribution of plasmid subtypes in different samples; (c) the origin of plasmids in relation to their source hosts; (d) the abundance profile of integrons; (e) the abundance of transposons; and (f) the abundance of insertion sequences. The colour gradients show how each kind of MGE or source host contributes to the overall MGE.

Significant associations with 18 ARGs each were observed for *S. aureus* and F. graminearum, suggesting that these bacteria may serve as pivotal reservoirs of multidrug Resistance (Li et al., 2024). It seems that these genes may be broadly dispersed across pathogenic hosts in mariculture environments, as three particular ARGs—macB, tetA(58), and evgS—were shown to be highly associated with all 19 pathogens evaluated.

Mariculture systems use antibiotics intensively, as shown by the prevalence of resistance genes for macrolides, tetracyclines, fluoroquinolones, and penicillins. Many of the top 20 pathogenic species may already have multidrug-resistant profiles, as they co-occur with a wide variety of ARGs. This is quite concerning because harmful bacteria pose a threat to human health, even when they are susceptible to a single antibiotic; when these bacteria develop Resistance to numerous medicines, treatment becomes much more difficult. There is a high probability of persistent resistance traits being inherited via vertical gene transfer, given the strong association between ARGs and pathogens. As a result, antibiotic resistance may be perpetuated within microbial communities after bacteria acquire it. Among the detected bacteria, *S. aureus* and F. graminearum stood out as clear indicators of multidrug Resistance (Yin et al., 2023). It is possible to use their presence in conjunction with other ARGs as a biological indicator to differentiate mariculture effluent from other types of water pollution. To sum up, mariculture waters serve as breeding grounds for novel MDR bacteria and viruses and as repositories for antibiotic resistance genes (Dai et al., 2023). Mariculture zones are a significant source of the increasing problem of antibiotic resistance in the environment because of the interconnectedness of ARGs, MGEs, and harmful bacteria.

Fourteen antibiotic resistance genes (ARGs) from seven different classes were shown to be highly related to insertion sequences and plasmids, according to analysis of co-occurrence patterns (Fig. 5b). An analysis of the relationships between insertion sequences and plasmids revealed that some ARGs, including arlS, baeS, cpxA, efrA, msbA, mtrA, patA, and tetA(58), are very mobile and may be horizontally transported

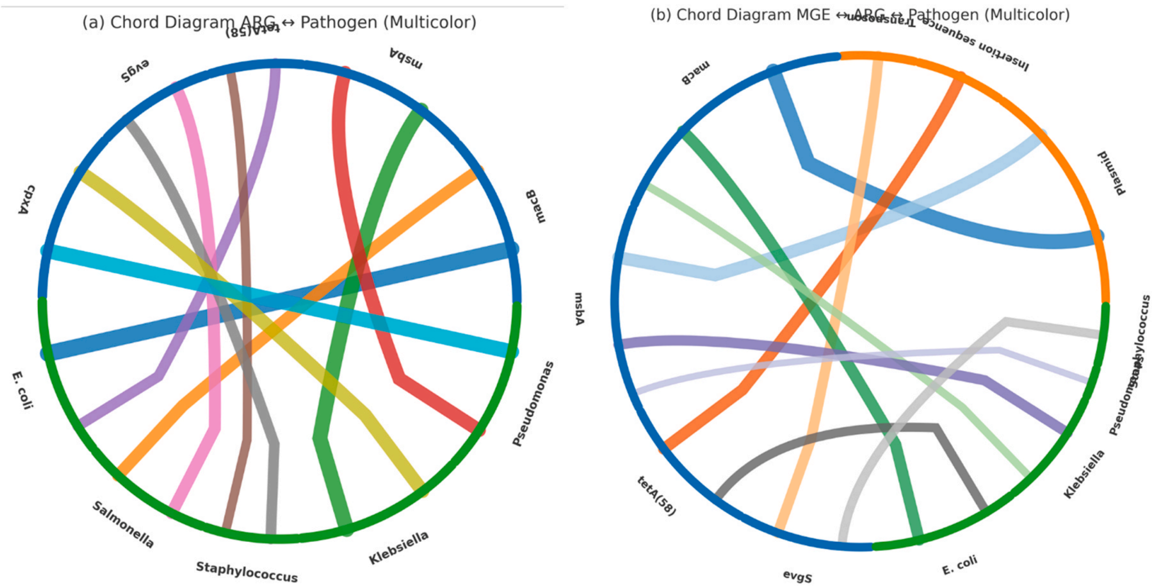


Fig. 5. shows the network of co-occurrences of (a) the top 20 pathogens with ARGs and (b) MGEs with both pathogens and ARGs.

across different species of bacteria. Plasmids and insertion sequences can facilitate the transmission of traits conferring Resistance to multiple drugs, as five such traits were associated with these elements (Hwang et al., 2025).

Notably, there was a significant association between these mobile genetic elements and critical pathogenic bacteria, including E. coli, *S. enterica*, *P. aeruginosa*, and V. cholerae, compared with other bacteria, indicating that these bacteria have a greater ability to acquire or spread ARGs. According to these connections, plasmids and insertion sequences are the primary mechanisms for horizontal transfer of ARGs in mariculture. It is highlylikely that harmful species in mariculture systems acquire ARGs from environmental bacterial populations, as the investigation also revealed that plasmids and insertion sequences were found alongside several hazardous bacteria (Gao et al., 2021). The possibility that these infections might covertly acquire resistance traits and then regain viability under favorable conditions is underscored by the fact that their HGT capacity persists even when bacteria reach unculturable states. The presence of organic pollutants, trace metals, and antibiotic residues in mariculture systems can increase bacterial stress responses, membrane permeability, and mechanisms that facilitate gene transfer, thereby worsening the spread of ARGs. Under these circumstances, ARGs may easily spread among microbial populations, including harmful bacteria (Shakoor et al., 2020).

These results show that the mariculture waters along the 5000 km coastline of South China are a significant reservoir and vector for antibiotic resistance. It is believed that multidrug-resistant pathogenomes are formed and disseminated via high-frequency HGT events, driven by strong connections among ARGs, mobile genetic elements, and pathogenic bacteria. The development of antibiotic-resistant bacteria and viruses is a significant cause for alarm in mariculture systems, given the strong link between these systems and human health, both through the ingestion of seafood and through environmental exposure (Yang et al., 2023). To mitigate this increasing threat to public health, there needs to be a stronger push to track biological pollutants and enhance mariculture wastewater treatment.

3.3. Microbiome increasing the proliferation potential of mariculture waters by producing virulence factors

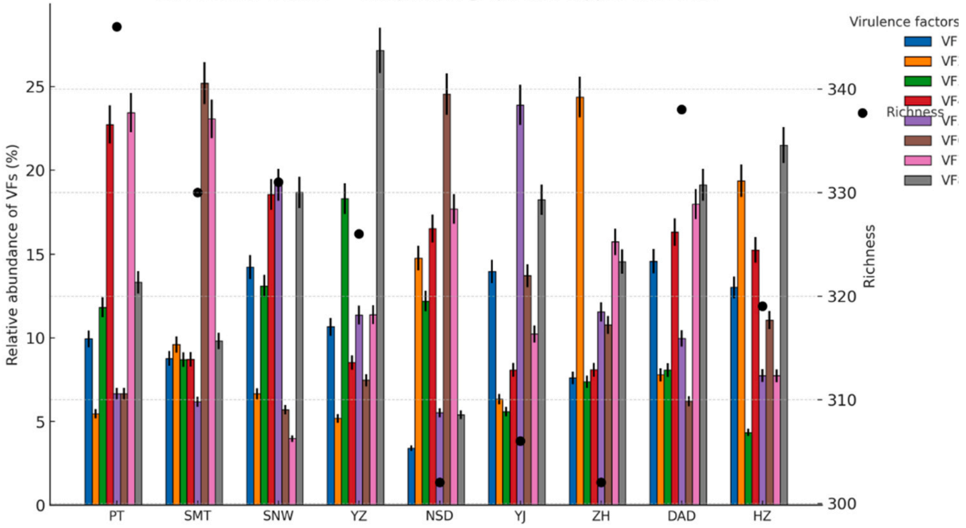
Assessing the number and source of virulence factors (VFs) allowed us to estimate the pathogenic potential of microbial communities in mariculture waters. A total of 908 proteins and 402 virulence factors were linked to human pathogenic pathways. All samples showed a high degree of consistency in the relative number and composition of VFs (Fig. 6a). The PHI-base and VFDB indicate that E. coli, *S. enterica*, and V. cholerae were the most common pathogenic bacteria and fungi that caused these VFs (Fig. 6b). According to the results of the Venn diagram, out of all the bacterial species linked to VFs that were identified using metagenomic and 16S rRNA sequencing, sample ZH had the most species in common, with nine. In comparison, the control groups PT and DAD had the fewest (Fig. 6c). Offensive virulence factors predominated at all locations, with the most enormous abundance seen for adherence-related components such as polar flagella, type IV pili, and capsules (Fig. 6a). Notable among the toxins were colibactin, beta-hemolysin, and Cya (Zhu et al., 2023).

Mariculture water samples had more diverse and abundant pathogenic VF-producing organisms than control locations. In a mariculture setting, the likelihood of developing antibiotic-resistant infections is significantly increased when these virulence determinants co-occur with ARGs and harmful bacteria. Mariculture water samples had a more varied and rich protein composition than control samples when analysed for proteins associated with human illness pathways (Scavo et al., 2022). The amounts of DNA (cytosine-5)-methyltransferase (DNMT1, dcm) were found to be higher in the SMT, ZH, and HZ samples. In every sample, pathways associated with cancer and bacterial infections took precedence, followed by those linked to antibiotic resistance. This research adds to the growing body of evidence that mariculture waters are breeding grounds for disease-causing organisms and may even include resistance-related proteins and virulence factors (Liu et al., 2023). The risk of antibiotic-resistant infections in coastal areas is further increased when virulence factors combine with ARGs and harmful bacteria.

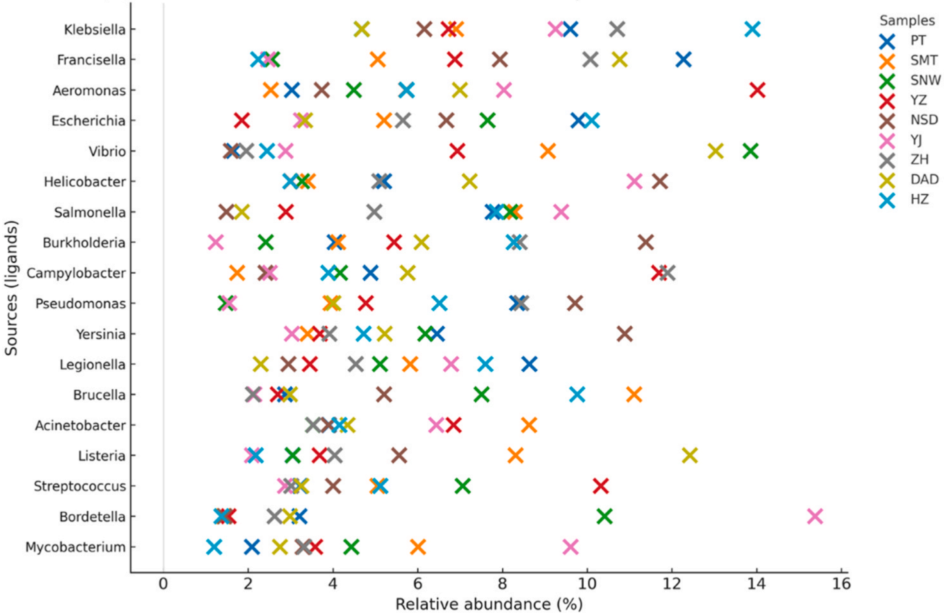
There is a diverse variety of virulence genes in the pathogenome of mariculture waters, as shown by the discovery of functional proteins associated with human pathogenic pathways and a large number of virulence factors (VFs). Mariculture waters have a high potential to serve as reservoirs for clinically significant pathogenomes, as these VFs were mainly associated with known human pathogens, including E. coli, M. tuberculosis, K. pneumoniae, S. pneumoniae, A. baumannii, L. monocytogenes, *S. enterica*, and V. cholerae.

The fact that VFs are so prevalent in bacterial hosts implies that these diseases have a higher potential for proliferation. Essential characteristics of pathogenic bacteria include Type IV pili, which aid in adhesion and immune evasion; colibactin, which causes DNA damage; and beta-

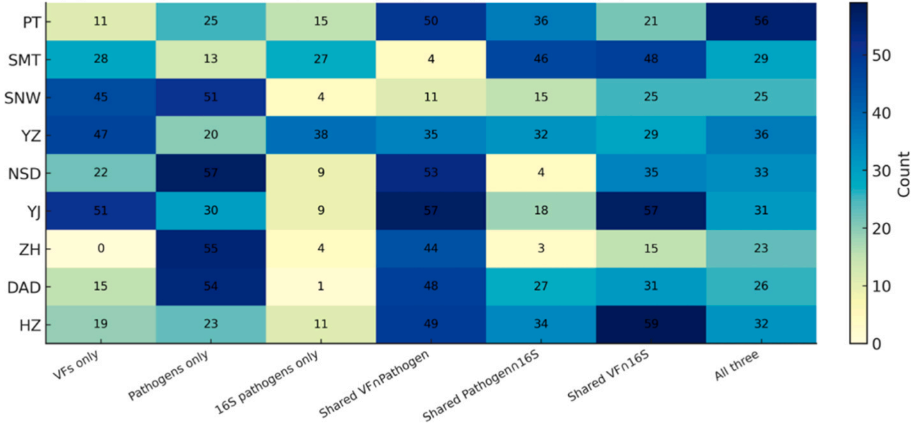
(a) Virulence factors — Grouped bar graph with capped error bars



(b) Sources of virulence factors — Cleveland plot with thick cross markers



(c) Overlap counts (VF / Pathogen / 16S) — Heatmap (Venn alternative)



	VFs only	Pathogens only	16S pathogens only	Shared VF∩Pathogen	Shared Pathogen∩16S	Shared VF∩16S	All three
PT	11	25	15	50	36	21	56
SMT	28	13	27	4	46	48	29
SNW	45	51	4	11	15	25	25
YZ	47	20	38	35	32	29	36
NSD	22	57	9	53	4	35	33
YJ	51	30	9	57	18	57	31
ZH	0	55	4	44	3	15	23
DAD	15	54	1	48	27	31	26
HZ	19	23	11	49	34	59	32

Fig. 6. (a) The frequency with which VFs are present in each sample; (b) the frequency with which bacteria derived from VFs are present in each sample; and (c) the frequency with which bacteria derived from VFs are present in both metagenomic and culturable pathogenic bacteria. Variegated colour coding indicates the relative abundance of various VFs or microorganisms produced from VFs, expressed as percentages.

hemolysin, which encourages biofilm development, cytotoxicity, and phagosome escape. Additionally, it was common to find Cya toxin, which has both haemolytic and dual adenylate cyclase activity, as well as iron-acquisition systems, which are essential for colonisation and toxin production. These characteristics increase bacteria's pathogenicity and help them survive in hosts. In sum, antibiotic-resistant, highly infectious bacteria may thrive in mariculture waters. There is a multiplied public health risk in these settings due to the presence of VFs, ARGs, and MGEs. Therefore, virulence factors, in addition to ARGs and harmful bacteria, should be included in future risk evaluations and monitoring in mariculture zones.

This research emphasizes the critical need for stronger antibiotic control in mariculture and improved monitoring. Policymakers should implement more stringent regulations on antibiotic use, including restricting prophylactic treatments and promoting sustainable alternatives such as probiotics, vaccines, and improved husbandry practices.

4. Conclusion

This study demonstrates that mariculture waters harbor diverse pathogenic communities, antibiotic resistance genes, and mobile genetic elements, with multidrug resistance genes predominating across sites. The data suggest that mariculture waters may facilitate horizontal gene transfer (HGT), including in bacteria that are difficult to culture, thereby contributing to the persistence and spread of resistance traits. These findings underscore the importance of improving management practices and monitoring systems to prevent contamination in marine ecosystems. Antibiotic resistance in mariculture has the potential to escalate into a serious public health and environmental emergency if left unaddressed. Urgent actions are required, including improved surveillance, reduced antibiotic use, and the implementation of best practices for water quality management. Policymakers should implement more stringent regulations on antibiotic use in mariculture, restricting prophylactic treatments and promoting sustainable alternatives such as probiotics, vaccines, and improved husbandry practices.

4.1. Limitations

This study represents a single-season snapshot, as all samples were collected between April and June 2021; therefore, potential seasonal variations in microbial community composition, antibiotic usage patterns, and horizontal gene transfer dynamics were not captured. To provide a fair interpretation of the results, it is necessary to note numerous limitations, despite the enormous scope of this research. Firstly, our capacity to resolve comprehensive genomic contexts may have been hindered by our reliance on short-read sequencing, even though we used high-throughput metagenomic sequencing and 16S rRNA studies to characterise the resistome, mobilome, and pathogenome. In particular, this limitation hinders our ability to understand gene mobility and pathogenic potential by preventing the precise linking of ARGs to specific hosts and mobile genetic elements. Our ability to validate phenotypic Resistance or to conduct experimental validation of pathogenicity and resistance gene expression is further limited, as we cannot cultivate the majority of the reported pathogens (197 out of 207).

Secondly, the research is still limited in terms of time, even if it covered a large geographic area (almost 5000 km of coastline). Seasonal changes in microbial community composition, trends in antibiotic use, and the dynamics of horizontal gene transfer may go unnoticed in the data, as they are snapshots in time. Even though they have a significant role in microbial ecology, environmental factors, including temperature, salinity, nutrient levels, and sediment composition, were not consistently quantified or included in our association studies. This might mask the fact that significant environmental factors influence differences in the resistome and mobilome. In addition, metagenomic annotation is database-dependent, which may lead to underestimation or bias, particularly for newly discovered or poorly understood genes and taxa. In maritime settings, where microbial diversity remains unexplored mainly, curated databases used for ARG and MGE assignment may miss emerging resistance determinants or mobile elements. Finally, although co-occurrence network analysis identified some interesting connections among infections, ARGs, and MGEs, it remains a correlational study. It cannot establish a causal relationship or confirm any actual gene transfer events.

4.2. Future policies implications

This study's findings provide substantial evidence for the role of mariculture waters in South China as reservoirs and possible amplification zones for multidrug-resistant bacteria. In light of these results, immediate and thorough governmental measures are required to reduce threats to marine ecosystems, food security, and human health. Implementing strict antimicrobial stewardship procedures in aquaculture activities is of the utmost importance. This involves monitoring how often and which antibiotics are used in mariculture, eventually eliminating them from non-therapeutic uses and encouraging people to use alternatives, such as probiotics and vaccinations, to manage diseases. To gather up-to-date information for risk assessments and policy changes, it would be beneficial to establish a nationwide surveillance system to track harmful bacteria, mobile genetic elements, and antibiotic resistance genes (ARGs) in mariculture settings. Collaboration across sectors, including aquaculture, public health, and maritime authorities, is essential for the successful implementation of such systems, which should include early warning systems to identify new resistance patterns.

Resistome and pathogenome profiles should be evaluated as part of the required environmental impact studies before aquaculture development, especially in areas with high levels of human involvement. Recirculating aquaculture systems (RAS), whether closed or semi-closed, may help reduce pollution levels and should be encouraged by policymakers. The results from South China highlight the need to share data and work together across borders to combat antibiotic resistance in water bodies. Antimicrobial Resistance (AMR) in mariculture requires regional and global cooperation through frameworks such as the FAO Codex Alimentarius, the World Health Organization's One Health approach, and the United Nations Sustainable Development Goals, as the ocean is not contained within boundaries. Finally, policymakers should invest in research and training programs to better understand marine resistomes and to fund the development of innovative biotechnological tools to detect and manage ARGs and resistant infections in water systems. Antibiotic resistance in mariculture has the potential to escalate into a serious public health and environmental emergency if authorities do not take aggressive measures to address the problem.

CRediT authorship contribution statement

Hao Cao: Conceptualization; Methodology; Formal analysis; Writing – original draft; Supervision. **Taicheng An:** Data curation; Software; Validation; Visualization; Writing – review & editing. **Yiwei Cai:** Investigation; Resources; Project administration; Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rsma.2026.104885.

Data Availability

The authors do not have permission to share data.

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