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DISTRIBUTION OF ANTIBIOTIC RESISTANCE GENES IN MARINE WATER AND SEDIMENTS IN AQUACULTURE AREAS IN KHANH HOA, VIETNAM

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ABSTRACT

The emergence of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) poses significant risks to ecological security and human health. Coastal areas affected by urban and aquaculture activities serve as critical reservoirs for pollutants, including ARGs. However, the data on the abundance and distribution of ARGs in marine sediments impacted by aquaculture activities in the East Sea, Vietnam, remain limited. This study investigated the distribution of ARGs across four aquaculture areas in the East Sea of Khanh Hoa province, Vietnam. The results revealed that sulfonamide-resistant genes, *sul1* and *sul2*, were present at all sampling sites (100%), with abundance ranging from 2.4×10^2 to 2.0×10^5 copies/ml in seawater and from 2.0×10^5 to 2.57×10^7 copies/g in sediments. Tetracycline-resistant genes, including *tetQ*, *tetM*, and *tetX*, were detected at most sampling sites, with abundances ranging from 1.29×10^1 to 6.16×10^6 copies/ml in seawater and from 1.64×10^4 to 1.04×10^8 copies/g in sediments. The vancomycin-resistant gene *(vanA)* exhibited significant abundance, ranging from 1.38×10^3 to 3.22×10^4 copies/ml in seawater and from 1.14×10^6 to 1.9×10^7 copies/g in sediments. Finally, integron class 1 (*intI1*) was detected at all sampling sites (100%) in both seawater and sediments, with abundances up to 6.86×10^5 copies/ml in seawater and 2.06×10^8 copies/g in sediments. Given aquaculture's significant role in Vietnam's economic growth, implementing effective technologies and national policies to manage and mitigate antimicrobial resistance in aquaculture environments is crucial.

Keywords: Antibiotic resistance, antibiotic resistant gene, aquaculture areas, integron class 1, Khanh Hoa, East Sea

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most pressing global public health threats, currently causing approximately 700,000 deaths per year, with projections suggesting this number could rise to 10 million annually by 2050 if no substantial interventions are made (Kraker *et al.*, 2016). The World Health Organization (WHO) identified AMR as one of the top 10 threats to global health, attributing much of this rise to the indiscriminate and excessive use of antibiotics in human medicine, agriculture, and aquaculture (WHO, 2020). The One Health approach, advocated by the WHO, Food and Agriculture Organization (FAO), and World Organization for Animal Health (WOAH), emphasizes the interconnectedness of human, animal, and environmental health in combating AMR (Pitt and Gunn, 2024). This framework recognizes the environment as a crucial reservoir and vector for the dissemination of antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB), enabling the movement of resistance factors between ecological compartments. Integron class 1 (*intI1*), a mobile genetic element, plays a key role in facilitating horizontal gene transfer of ARGs within bacterial communities (Gillings *et al.*, 2015). Oceans, in particular coastal marine environments, represent significant reservoirs of resistant bacteria and ARGs due to the convergence of contaminants, including pharmaceutical residues, from terrestrial and marine sources (Hatosy and Martiny, 2015). The mixing of bacteria from these sources, coupled with increased anthropogenic pressures, facilitates the selection of resistant strains, posing serious threats to both human health and marine biodiversity (Siri *et al.*, 2023). Despite these global concerns, research efforts have predominantly focused on resistance in terrestrial environments, leaving marine ecosystems comparatively understudied (Prestinaci *et al.*, 2015).

Vietnam faces significant challenges in addressing AMR (Quang Huy *et al.*, 2023; Van Anh *et al.*, 2023), driven in large part by the widespread use of antibiotics in both human healthcare and animal production (Mitchell *et al.*, 2020; WHO, 2023). A recent study highlighted that approximately 70% of antimicrobial consumption in Vietnam occurs within the animal production sector, which significantly contributes to the emergence and spread of resistance across both agricultural and aquaculture environments (Phu *et al.*, 2022). As one of the world’s largest aquaculture producers, Vietnam's aquaculture industry operates with limited regulatory oversight, exacerbating the proliferation of resistant bacteria in these settings. Although research on terrestrial and estuarine AMR in Vietnam has increased, information on ARGs in marine water and sediments remains limited. In particular, data on the abundance and distribution of ARGs in the marine environments influenced by aquaculture activities in the East Sea of Vietnam are still scarce.

Khanh Hoa, located along Vietnam's southern central coast, serves as a critical case study for investigating AMR in marine environments. The region is heavily influenced by urban wastewater and aquaculture effluent, which introduce a variety of antibiotics (such as tetracycline, trimethoprim, rifampicin, nifuroxazide, and colistin) into the marine ecosystem (Hedberg *et al.*, 2018). A recent study found *sul1, sul2, sul3*, and *intI1* widely distributed in seawater and sediment in Nha Trang Bay, suggesting that this contamination provides an environment conducive to the emergence and spread of multidrug-resistant bacteria and ARGs (Tran *et al.*, 2025). Given the proximity of aquaculture farms to densely populated urban areas and the region's heavy reliance on seafood, Khanh Hoa Sea represents a notable risk for the transmission of resistant bacteria to humans, underscoring the need for continuous monitoring of AMR across different geographical regions. This study, therefore, aimed to assess the distribution and abundance of ARGs in the water and sediments of Khanh Hoa Sea belonging to East Sea, Vietnam (https://chinhphu.vn/). Our findings may enhance understanding of the role marine ecosystems play in the global dissemination of AMR and emphasize the necessity for stricter regulations in aquaculture and environmental management.

MATERIALS AND METHODS

Study area

In November 2023, a total of 24 environmental samples (12 surface water and 12 sediments) were collected from four key locations in the Khanh Hoa Sea (East Sea, Vietnam): Hon Mieu (12.19253°N, 109.21825°E), Nha Phu (12.39400°N, 109.22093°E), Bich Dam (12.18977°N, 109.31550°E), and Cam Ranh (11.92683°N, 109.18527°E). These sites are chosen to represent typical residential exposures to diverse domestic livestock and wildlife species but different environmental settings (e.g., closeness to coral reefs). Most of these farms have been interviewed in a precedent study of our consortium (Hedberg *et al.*, 2018), increasing the probability of acceptance of enrollment for this new survey.

Sample collection

At each sampling station, three surface water samples were collected at a depth of 15 cm below the surface using 500 ml sterile polypropylene bottles, while three sediment samples were obtained from the top 5 cm of the sediment layer using a Van Veen grab sediment sampler. The collected sediment was then transferred into 50 ml Falcon tubes for preservation. All samples were promptly placed in an insulated cooler with ice packs to maintain their integrity and transported to the laboratory at the Institute of Oceanography (IO) in Nha Trang city for subsequent analysis.

Environmental DNA extraction

For water samples, 1.5 liters were filtered through 0.2 μm cellulose acetate membrane filters (Sartorius Biotech, France) to concentrate bacterial cells. The filters were then subdivided into small sections for DNA extraction using the DNeasy PowerSoil Pro kit (Qiagen, Germany) (https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/microbial-dna/dneasy-powersoil-pro-kit). For sediment samples, 200 mg of sediment was processed for DNA extraction using the same kit. Following the manufacturer's protocol, DNA was extracted from sediment and filter samples using a combination of chemical lysis and mechanical homogenization with zirconium bead beating. The crude lysate was then subjected to inhibitor removal before being mixed with a DNA binding solution and passed through a silica spin column. After a two-step wash, DNA was eluted using 10 mM Tris buffer. The quality and quantity of the extracted DNA were evaluated by agarose gel electrophoresis, and DNA concentration was measured by a NanoDrop™ 2000/2000c spectrophotometer (Thermo Fisher

Scientific, USA). All the extracted DNA was stored at -20ºC for further analysis.

Abundance of antibiotic resistance genes

To assess the presence and abundance of ARGs in the marine environment of Khanh Hoa Sea, eight target ARGs were analyzed. These genes are associated with resistance to sulfonamides (*sul1*, *sul2*), tetracyclines (*tetX*, *tetM*, *tetQ*), and glycopeptides (*vanA*). In addition, the 16S rRNA gene, used as a marker for bacterial density, and integron class 1 (*intI1*), a mobile genetic element linked to the dissemination of ARGs and virulence factors, were also quantified. Quantitative PCR (qPCR) analysis was conducted using the FastGene 2x IC Green qPCR Universal Mix on the QuantStudio 5 system (Thermo Fisher Scientific, USA). Primer sequences and qPCR conditions for each target gene have been previously described (Tran *et al.*, 2025). Standard curves for absolute quantification were generated by performing qPCR on serial 10-fold dilutions for each gene, with efficiencies ranging from 91.5% to 108.9% and correlation coefficients ($R^2 > 0.91$), ensuring accurate and reliable quantification. Melting temperatures (Tm) of the amplified products were assessed to confirm the specificity of the qPCR assays. The copy numbers of the target genes were calculated based on the calibration curves and expressed as copies per milliliter for water samples and copies per gram for sediment samples. The absolute abundance of ARGs, *intI1*, and the 16S rRNA gene was determined to evaluate the prevalence of these genes and the potential for horizontal gene transfer (HGT) within the marine microbial community.

Data analysis

To assess variations in both the relative and absolute abundances of ARGs across different substrates and sampling sites, we utilized the Kruskal-Wallis test, followed by Dunn's post-hoc test for nonparametric data. For parametric data, Levene's test was applied to examine homogeneity of variances, with a subsequent Tukey's post-hoc test for pairwise comparisons. To control for false discovery rates, p-values were adjusted using the Benjamini-Hochberg procedure. Additionally, differences in the total absolute abundance of ARGs across substrates and transects were further assessed using Permutational Multivariate Analysis of Variance (PERMANOVA), employing the Bray-Curtis distance matrix to measure dissimilarities. All statistical analyses were conducted using R software, version 4.2.0.

RESULTS AND DISCUSSION

Distribution and abundance of bacteria, ARGs, and integron class 1 in seawater

Seawater samples exhibited bacterial abundances ranging from 4.47×10^5 to 1.37×10^8 copies/ml (Table 1). The highest mean bacterial abundance was observed in Bich Dam, while the lowest was in Hon Mun (Figure 1). For sulfonamide-resistant genes, both *sul1* and *sul2* were detected across all sampling sites (100%), with *sul1* abundance ranging from 2.40×10^2 to 1.94×10^5 copies/ml, and *sul2* from 3.29×10^3 to 2.00×10^5 copies/ml. The mean abundance of *sul2* was generally higher than that of *sul1*. Notably, the highest mean abundances for both genes were found in Cam Ranh, with *sul1* abundance lowest in Nha Phu, and *sul2* abundance lowest in Hon Mun (Figure 1). Regarding tetracycline-resistant genes, *tetQ* was detected in all 12 sampling sites (100%), *tetM* in 11/12 sites (91.7%), and *tetX* in 9/12 sites (75%) (Table 1). The absolute abundance of *tetQ* ranged from 3.71×10^2 to 6.16×10^6 copies/ml, *tetM* from 1.29×10^1 to 1.62×10^4 copies/ml, and *tetX* from 5.90×10^3 to 6.71×10^4 copies/ml. The mean abundance of *tetQ* was highest, followed by *tetX* and *tetM* (Figure 1). The abundance of *vanA* ranged from 1.38×10^3 to 3.22×10^4 copies/ml, showing no significant variation between transects. It was most prevalent in Cam Ranh and Bich Dam, with lower levels in Hon Mun. Finally, *intI1*, a key integron-associated gene, was detected in all sampling sites (100%) with an abundance ranging from 1.30×10^4 to 6.86×10^5 copies/ml. The mean abundance of *intI1* showed no significant variation across the four transects.

Table 1. The absolute abundance of bacteria, integron class 1 and ARGs in seawater

Sampling location	**Sampling station**	**Absolute abundance of target genes in seawater (copies/ml)**							
		16S rRNA	*sul1*	*sul2*	*tetQ*	*tetX*	*tetM*	*vanA*	*IntI1*
Nha Phu	NP_W_4	$5.20x10^{5}$	$4.16x10^{2}$	$2.10x10^{4}$	$3.71x10^{2}$	$1.35x10^{4}$	ND	$6.43x10^{3}$	$5.85x10^{4}$
	NP_W_5	$2.34x10^{7}$	$4.36x10^{2}$	$1.01x10^{4}$	$9.77x10^{2}$	ND	$1.29x10^{1}$	$1.78x10^{3}$	$1.67x10^{4}$
	NP_W_6	$9.27x10^{5}$	$2.50x10^{2}$	$3.78x10^{4}$	$9.95x10^{2}$	$1.55x10^{4}$	$8.05x10^{1}$	$6.52x10^{3}$	$5.98x10^{4}$
Hon Mun	HM_W_4	$4.47x10^{5}$	$1.49x10^{3}$	$4.97x10^{3}$	$7.63x10^{3}$	ND	$3.81x10^{1}$	$3.13x10^{3}$	$2.26x10^{4}$
	HM_W_5	$5.62x10^{5}$	$2.40x10^{2}$	$5.63x10^{3}$	$7.08x10^{3}$	ND	$1.89x10^{2}$	$1.60x10^{3}$	$6.86x10^{5}$
	HM_W_6	$8.34x10^{5}$	$1.63x10^{3}$	$3.29x10^{3}$	$9.57x10^{2}$	$5.90x10^{3}$	$6.22x10^{1}$	$1.38x10^{3}$	$1.30x10^{4}$
Bich Dam	BD_W_4	$2.69x10^{7}$	$2.48x10^{3}$	$1.33x10^{4}$	$9.68x10^{3}$	$1.49x10^{4}$	$1.02x10^{3}$	$1.14x10^{4}$	$1.88x10^{4}$
	BD_W_5	$1.37x10^{8}$	$1.87x10^{3}$	$1.44x10^{5}$	$5.36x10^{4}$	$6.02x10^{4}$	$1.12x10^{3}$	$2.68x10^{4}$	$3.29x10^{4}$
	BD_W_6	$3.35x10^{7}$	$7.73x10^{2}$	$4.37x10^{4}$	$7.10x10^{3}$	$3.43x10^{4}$	$3.36x10^{2}$	$2.36x10^{4}$	$1.36x10^{4}$
Cam Ranh	CR_W_4	$1.79x10^{7}$	$1.94x10^{5}$	$2.00x10^{5}$	$2.30x10^{3}$	$6.71x10^{4}$	$1.36x10^{2}$	$1.60x10^{4}$	$4.00x10^{4}$
	CR_W_5	$3.39x10^{7}$	$5.44x10^{4}$	$4.84x10^{4}$	$6.16x10^{6}$	$4.45x10^{4}$	$1.62x10^{4}$	$1.81x10^{4}$	$1.54x10^{5}$
	CR_W_6	$2.51x10^{7}$	$1.73x10^{5}$	$1.70x10^{4}$	$1.46x10^{3}$	$1.48x10^{4}$	$2.76x10^{1}$	$3.22x10^{4}$	$1.96x10^{4}$

ND: Not detected

Previous studies highlighted the heavy and often unregulated use of antibiotics in aquaculture systems across Asia (Hedberg *et al*., 2018; Siri *et al*., 2023; Zhang *et al*., 2022). The persistence of these antibiotics in aquatic environments - particularly those that are non-biodegradable - can lead to long-term reservoirs of resistance genes. These studies also emphasized how these ARGs can accumulate in sediments and biota, and are then disseminated via HGT mechanisms, especially through mobile genetic elements such as integrons (e.g., *intI1*), which were detected in high abundance in our study. The genes *sul1* and *sul2* were particularly prominent, as observed in our study. The similarities in ARG profiles and bacterial abundances strongly suggest that coastal waters, especially those influenced by aquaculture discharge, serve as critical hotspots for ARG proliferation. A recent study investigated the occurrence and distribution of ARGs in seawater samples collected from Nha Trang Bay, Vietnam, revealing the widespread presence of eight ARGs, including *sul1*, *sul2*, *sul3*, *tetQ*, *tetB*, *mecA*, *blaVIM*, and *blaKPC* (Tran *et al.*, 2025). Notably, *intI1* was detected in 95.8% of the samples, with a mean abundance of 2.9×10^5 copies/ml. The study also identified significant correlations between the presence of *sul3*, *tetB*, *blaVIM*, *blaKPC*, and *intI1* and environmental factors such as bacterial density, chlorophyll A concentrations and phosphorus levels. These findings highlight that polluted environments facilitate bacterial growth and promote the propagation of ARGs through horizontal gene transfers. Similarly, a study on the distribution of ARGs in seawater from the China Sea reported that the total absolute abundance of ARGs ranged from 2.1×10^3 to 2.3×10^4 copies/ml, while ARGs associated with MGEs ranged from 2.2×10^3 to 1.8×10^4 copies/ml (Lu *et al.*, 2024). Notably, multidrug-resistant bacteria, including foodborne pathogens such as *E. coli*, *Aeromonas*, and *Vibrio*, were also identified in both studies (Lu *et al.*, 2024; Tran *et al.*, 2025). These findings underscore the urgent need for national policies and technological interventions to combat AMR in aquaculture in these regions.

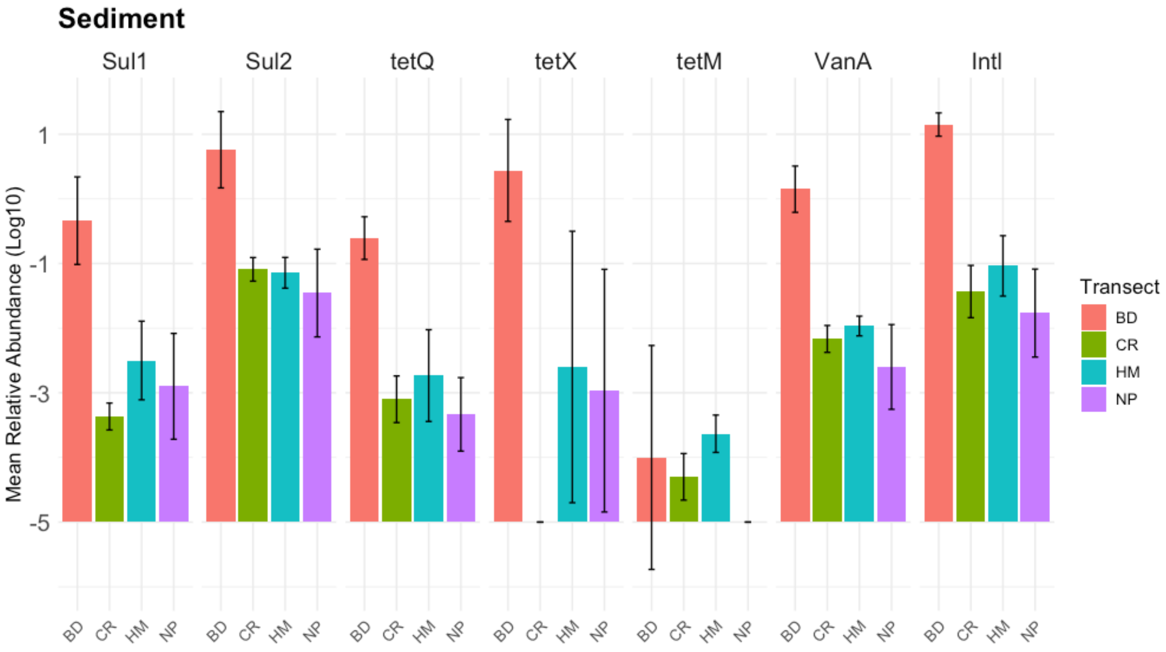

Figure 1. The log10 of mean absolute abundance of targeted genes in seawater across sampling stations: BD - Bich Dam; CR - Cam Ranh; HM - Hon Mieu; NP - Nha Phu.

Distribution and abundance of bacteria, ARGs, and integron class 1 in sea sediments

Sea sediment samples exhibited a broad range of bacterial abundance, from 8.48 × 10^6 to 3.21 × 10^9 copies/g (Table 2). Notably, the highest abundance was observed in Nha Phu, while Bich Dam showed the lowest levels. Both sulfonamide-resistant genes, *sul1* and *sul2*, were detected at all sites (100%). The abundance of *sul1* ranged from 2.00 × 10^5 to 2.57 × 10^7 copies/g, whereas *sul2* ranged from 4.35 × 10^6 to 4.35 × 10^6 copies/g (Table 2). Remarkably, *sul2* consistently exhibited a higher mean abundance than *sul1* (Figure 2). The highest concentrations of both genes were found in Bich Dam, while Hon Mun showed the lowest abundance.

In line with water samples, *tetQ* was universally detected across all sediment samples (100%), whereas *tetM* and *tetX* were present in only 58.3% (7 out of 12) of the samples (Table 2). Interestingly, *tetX* was absent in all Cam Ranh samples, and *tetM* was not detected in any Nha Phu samples. The abundance of *tetQ* varied from 9.39 × 10^4 to 8.12 × 10^6 copies/g, *tetX* ranged from 5.28 × 10^6 to 1.04 × 10^8 copies/g, and *tetM* ranged from 1.64 × 10^4 to 7.83 × 10^4 copies/g. Notably, *tetQ* and *tetX* exhibited the highest mean abundances in Bich Dam, while *tetM* showed comparable mean levels in both Nha Phu and Hon Mun.

VanA was detected at all sampling transects (100%), with abundances ranging from 1.14 × 10^6 to 1.90 × 10^7 copies/g (Table 2). The highest mean abundance was recorded in Bich Dam, while Hon Mun had the lowest levels (Figure 2). Similarly, *intI1* was universally detected (100%) across all transects, with abundance ranging from 3.79 × 10^6 to 2.06 × 10^8 copies/g. The highest levels of *intI1* were observed in Bich Dam, while Hon Mun exhibited the lowest abundance (Table 2).

Similarly, high abundances of *sul1*, *sul2*, *sul3*, *tetQ*, *tetB*, and *mecA* were detected in sediment samples from Nha Trang Bay, with mean absolute abundances ranging from 5.4 × 10^4 to 2.0 × 10^7 copies/g (Tran *et al*., 2025). Additionally, the *intI1* gene was present in sediment samples, with abundance levels ranging from 1.66 × 10^6 to 3.96 × 10^7 copies/g. The average absolute abundance of *intI1* in these sediment samples was 8.8 × 10^6 copies/g. Consistent with these findings, the high concentrations of ARGs observed in our study suggest that *intI1* plays a significant role in facilitating the spread of ARGs within bacterial communities in all sea areas of Khanh Hoa province.

Correlation of distribution of ARGs with bacteria, integron class 1, and environmental substrates

Consistent with previous studies, bacterial and integron abundance in sediment samples was significantly higher than in water samples ($p < 0.05$) (Figure 3). Similarly, the prevalence of *sul*1, *sul*2, *vanA*, and *tetQ* was notably greater in sediments than in water samples. This widespread prevalence across all sampling transects indicates extensive transmission and dissemination of these ARGs throughout the Khanh Hoa Sea. Sediments provide a nutrient-rich environment that supports microbial growth, and they also act as a stable reservoir for pollutants such as antibiotics, heavy metals, and other contaminants (Rodgers *et al*., 2019).

Table 2. The absolute abundance of bacteria, integron class 1 and ARGs in sea sediments

Sampling location	**Sampling station**	**Absolute abundance of target genes in sea sediments (copies/g)**							
		16S rRNA	*sul1*	*sul2*	*tetQ*	*tetX*	*tetM*	*vanA*	*Intl1*
Nha Phu	NP_S_4	3.21x10^{9}	2.31x10^{6}	1.89x10^{7}	9.79x10^{5}	7.65x10^{6}	ND	1.70x10^{6}	1.75x10^{7}
	NP_S_5	2.65x10^{8}	2.79x10^{6}	3.61x10^{7}	5.30x10^{5}	1.34x10^{7}	ND	2.58x10^{6}	2.67x10^{7}
	NP_S_6	1.29x10^{9}	3.43x10^{5}	6.52x10^{7}	2.29x10^{5}	ND	ND	4.01x10^{6}	1.22x10^{7}
Hon Mun	HM_S_4	2.89x10^{8}	2.00x10^{5}	2.35x10^{7}	9.39x10^{4}	5.28x10^{6}	4.40x10^{4}	2.37x10^{6}	1.83x10^{7}
	HM_S_5	1.06x10^{8}	6.45x10^{5}	4.35x10^{6}	8.28x10^{5}	ND	1.64x10^{4}	1.17x10^{6}	3.79x10^{6}
	HM_S_6	6.97x10^{7}	5.79x10^{5}	8.14x10^{6}	1.66x10^{5}	5.57x10^{6}	3.90x10^{4}	1.14x10^{6}	2.16x10^{7}
Bich Dam	BD_S_4	1.42x10^{7}	2.23x10^{6}	2.92x10^{8}	8.12x10^{6}	1.04x10^{8}	ND	3.61x10^{7}	1.25x10^{8}
	BD_S_5	9.41x10^{6}	2.57x10^{7}	6.64x10^{7}	1.24x10^{6}	7.92x10^{7}	ND	1.90x10^{7}	2.06x10^{8}
	BD_S_6	8.48x10^{6}	1.89x10^{6}	1.15x10^{7}	1.68x10^{6}	8.86x10^{6}	7.83x10^{4}	4.67x10^{6}	1.30x10^{8}
Cam Ranh	CR_S_4	9.32x10^{8}	2.36x10^{5}	4.78x10^{7}	2.69x10^{5}	ND	1.93x10^{4}	3.96x10^{6}	1.29x10^{7}
	CR_S_5	5.96x10^{8}	3.95x10^{5}	5.83x10^{7}	6.16x10^{5}	ND	6.18x10^{4}	6.46x10^{6}	3.56x10^{7}
	CR_S_6	4.51x10^{8}	2.37x10^{5}	4.84x10^{7}	6.41x10^{5}	ND	2.60x10^{4}	3.98x10^{6}	2.54x10^{7}

ND: Not detected

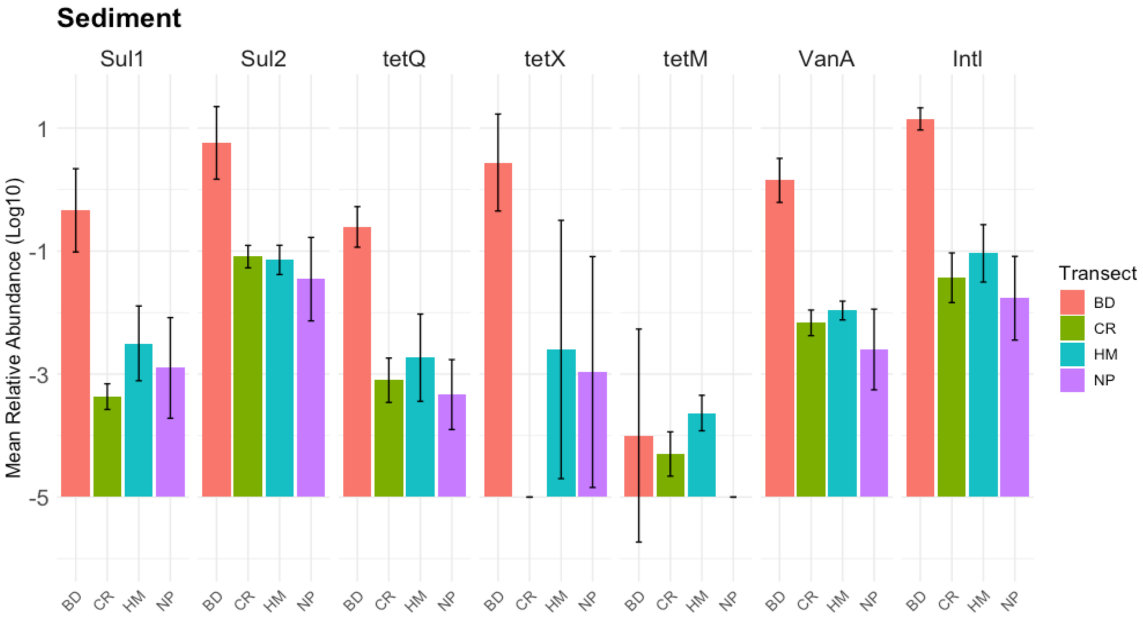

Figure 2. The mean absolute abundance of targeted genes in sea sediments across sampling stations: BD - Bich Dam; CR - Cam Ranh; HM - Hon Mieu; NP - Nha Phu.

These factors create optimal conditions for the selection of ARB and ARGs. Notably, this region impacted by urbanization and aquaculture activities exhibit a rapid selection process for ARB and ARGs (Tran *et al*., 2025). Although the mean abundances of *tetX* and *tetM* did not significantly differ between water and sediment samples, their prevalence was notably higher in samples from Nha Phu and Bich Dam compared to those from Hon Mun and Cam Ranh (Figure 3). Furthermore, the high abundance of *intI1* in both water and sediment samples across all transects highlights its crucial role in the multiplication and horizontal gene transfer of ARGs (Gillings *et al*., 2015).

Overall, areas with dense aquaculture activities, such as Cam Ranh, Nha Phu, and Bich Dam, exhibited higher bacterial abundance, ARGs, and integron prevalence than the more conserved region of Hon Mun. This variation suggests that local anthropogenic activities and environmental factors influence ARG prevalence. Similar trends have been observed in other aquatic environments, where human activities contribute significantly to the dissemination of ARGs (Zhang *et al*., 2022). Despite regional differences, the consistent ARG profiles across the sampling sites underscore the role of sediments and water bodies as reservoirs that facilitate the broader dissemination of ARGs (Tran *et al*., 2025; Wang *et al*., 2024).

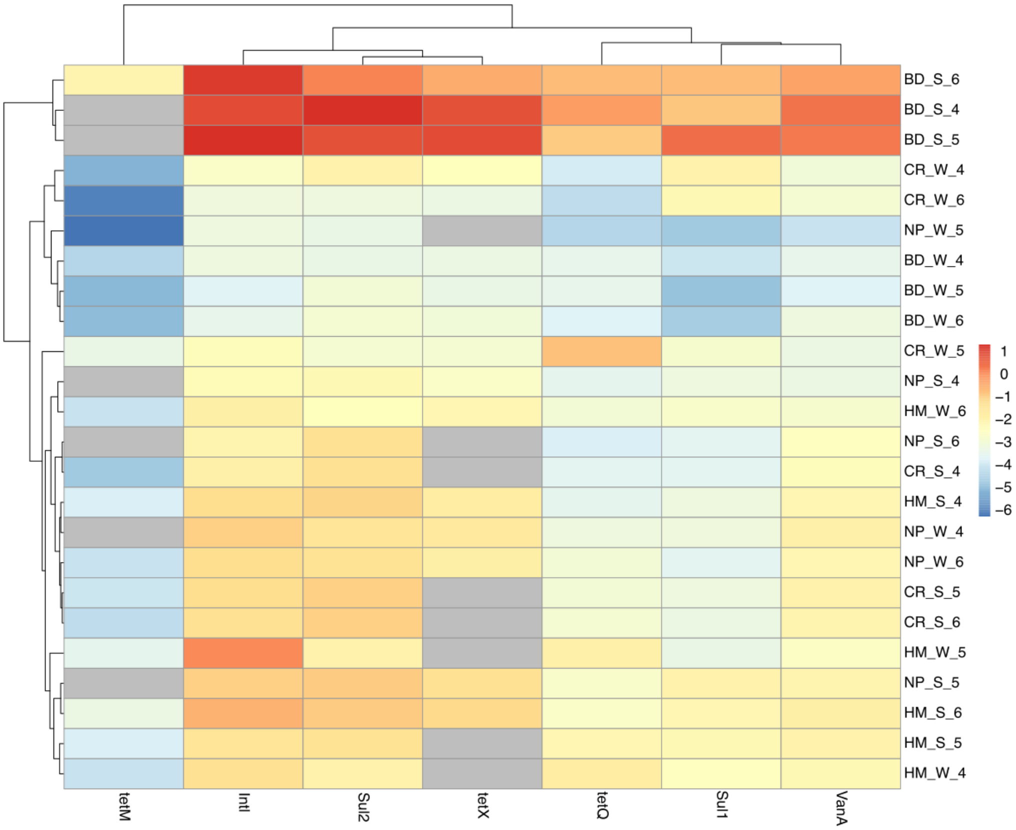

Figure 3. The absolute abundance of target genes in the marine environments of Khanh Hoa Sea, East Sea, Vietnam.

The Principal Coordinate Analysis (PCoA) revealed a distinct separation between seawater and sediment samples, with sediment samples clustering separately from water samples (Figure 4). This segregation underscores the influence of environmental matrices on the distribution of ARGs. The PERMANOVA analysis showed that, while geographic location (sampling area) had no significant effect on ARG profiles ($R^2 = 0.13$, $p = 0.42$), the type of sample (water vs. sediment) had a substantial impact ($R^2 = 0.64$, $p = 0.0009$) (Figure 4). This result can be explained by the fact that although ARGs were more abundant in sediment due to the concentration of bacteria, water flow was more dynamic, allowing the ARGs or ARBs to be distributed widely in the water environment (Deng *et al*., 2020; Lu *et al*., 2024; Tran *et al*., 2025; Wang *et al*., 2024). Moreover, the excessive amount of antibiotics in aquaculture activity was also directly released into the water environment through the metabolism of domesticated aquatic animals or unconsumed antibiotics, promoting the proliferation of ARGs within this environment. These findings consolidate with other studies about the critical role of environmental compartments in determining the abundance and dissemination of ARGs (Tran *et al*., 2025; Wang *et al*., 2024).

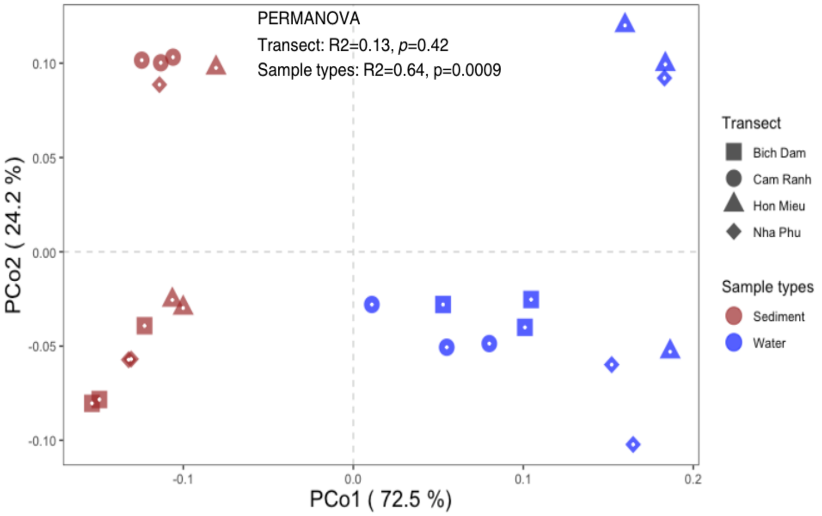

Figure 4. PCOA of samples separated by transects (Bich Dam, Cam Ranh, Hon Mieu, and Nha Phu) and sample types (Water and Sediment), using the ARGs abundance profile of each sample.

CONCLUSION

This study demonstrates the widespread presence and high abundance of ARGs and *intI1* in seawater and sediment samples from Khanh Hoa Sea (East Sea, Vietnam). Sulfonamide and tetracycline resistance genes were consistently detected across all sampling sites. The elevated abundance of *intI1*, especially in surface waters, suggests a strong potential for horizontal gene transfer and regional dissemination of AMR. These findings highlight the significant influence of anthropogenic activities, particularly aquaculture, on ARG spread in marine environments. To mitigate this issue, stricter regulations on antibiotic use in aquaculture are urgently needed.

Additionally, long-term monitoring programs for ARGs and mobile genetic elements in coastal ecosystems should be established. Collaborative efforts among scientists, policymakers, and local communities are essential to control and reduce AMR risks.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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