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INVESTIGATION OF POTENTIALLY PATHOGENIC MULTIDRUG-RESISTANT BACTERIA AND ANTIBIOTIC-RESISTANCE GENES IN THE NHA TRANG SEA, VIETNAM

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ABSTRACT

This study investigated the circulation of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in the Nha Phu and Bich Dam areas, the Nha Trang Sea, Vietnam, a region experiencing considerable environmental stress due to urbanization and tourism. Multidrug-resistant bacteria including *Escherichia coli*, *Enterobacter hormaechei*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Vibrio alginolyticus*, *Bacillus cereus*, and *Micrococcus luteus* were detected in sediment and water samples. The abundance of marine microbiome was from 3.8×10^9 to 1.5×10^9 copies/mL in surface water samples and in the sediment and from 9.9×10^7 to 2.6×10^9 copies/g in sediments. Among the four target ARGs, the *sul*1 and *sul*2 genes associated with sulfonamides resistance were detected in both water and sediment samples ranging from 2.3×10^0 to 4.5×10^3 copies/mL, and from 2.0×10^3 to 4.7×10^5 copies/g in water and sediment samples, respectively. For tetracycline resistance, *tet*Q and *tet*M were detected in 60% and 100% studied samples. The abundance of these genes was up to 1.7×10^2 copies/mL in water samples, and 1.1×10^5 copies/g in sediments. The class 1 integron-integrase gene *int*I1 displayed from 6.2×10^2 to 2.6×10^3 copies/mL and from 4.8×10^4 to 8.2×10^5 copies/g in water and sediment samples, respectively. Our findings emphasize the risk of ARB and their associated ARGs being transmitted in the marine environments of the Nha Trang Sea through the assistance of mobile genetic elements.

Keywords: Antimicrobial resistance, antimicrobial resistance genes, class 1 integron-integrase gene, Nha Trang, marine environment, multidrug-resistant bacteria.

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INTRODUCTION

Antimicrobial resistance (AMR) poses a major challenge to global health and sustainable development, threatening progress toward at least six of the seventeen United Nations Sustainable Development Goals (SDGs) (Aslam et al., 2024). The economic burden of AMR on healthcare systems is substantial, as it leads to more complex treatments, increased hospital admissions, and prolonged stays. Beyond its impact on human health, AMR jeopardizes food security and safety by compromising animal health and agricultural productivity. Without effective intervention, antibiotic-resistant infections could result in approximately 10 million deaths annually and an economic loss of up to USD 100 trillion by 2050 (De Kraker et al., 2016).

Aquatic ecosystems represent a hotspot for the proliferation, acquisition, and spread of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs) in natural environments and human community (Schar et al., 2021; Suyamud et al., 2024; Wang et al., 2021). The One Health concept identifies marine and freshwater environments impacted by human activities as critical hubs for the spread of AMR (Franklin et al., 2024). These ecosystems not only harbor ARB but also facilitate the exchange of ARGs, accelerating their dissemination. Among aquatic environments, sea coastal areas receive contaminants and effluents from multiple sources such as urban rivers, urban sewages, wastewater treatment plants, tourism, and aquaculture activities (Adenaya et al., 2024; Tran et al., 2025; Xu et al., 2023). Furthermore, mobile genetic elements (MGEs) such as plasmids, prophages, and transposons have become one of the most important factors facilitating the acquisition and exchange of ARGs through horizontal gene transfers (HGTs) (Fu et al., 2022; Tokuda and Shintani, 2024). Thus, determining the abundance of potential pathogenic bacteria and MGEs in the sea coast environments is crucial to monitoring the risk of AMR transmission and spread in the community.

Vietnam, a recognized hotspot for AMR emergence and transmission, has identified AMR as a critical public health threat (Torumkuney et al., 2022). However, it is significant gaps in systematic AMR surveillance across the country. Current surveillance efforts primarily focus on healthcare settings, yet hospital-associated AMR represents only a fraction of the total burden (Phu et al., 2022; Torumkuney et al., 2022; Vu et al., 2021). The lack of comprehensive monitoring in community settings, livestock, and the environment underscores the urgent need for a coordinated, multisectoral approach to AMR surveillance and mitigation. Viet Nam has an extensive coastline of 3,260 km, stretching from Mong Cai in the North to Ha Tien in the Southwest, with territorial waters extending eastward and southeastward into the East Sea (https://vietnamembassy-usa.org/vietnam/geography). The country's coastal regions are rich in natural resources and hold significant economic value. However, rapid urbanization, tourism, and intensive aquaculture have led to increasing anthropogenic pressures, making these areas potential reservoirs for the emergence and spread of ARB and ARGs through human activities and food chains.

Nha Trang, one of Vietnam's largest and most well-known coastal cities, is facing severe marine pollution (Fruergaard et al., 2023; Hedberg et al., 2018). The city's wastewater infrastructure includes five sewers discharging directly into the sea, five flowing into the Cai River, and three into the Quan Truong River, all of which contribute to the escalating pollution in Nha Trang Bay. Additionally, hundreds of floating aquaculture farms operate within the bay, further exacerbating environmental degradation. The widespread use of antibiotics in aquaculture has intensified the selection and proliferation of ARB, posing ecological and public health risks in the region (Nguyen et al., 2020; Pham et al., 2018; Tran et al., 2025). In this context, the recent study aimed to investigate the circulation of ARB and ARGs in the Nha Trang Sea. The findings in this study can provide a picture of the role of environmental

reservoirs of resistance, transfer potential, and relevant pathways in the emergence and dissemination of ARB and ARGs in sea coastal areas of Vietnam.

MATERIALS AND METHODS

Sample collection

Water and sediment samples were systematically collected over a two-week period in July 2023 from three locations in Nha Phu area (NP1: 12.397150^{o}, 109.221167^{o}; NP2: 12.397717^{o}, 109.217317^{o}; NP3: 12.394517^{o}, 109.217267^{o}) and three locations in Bich Dam area (BD1: 12.190285^{o}, 109.317917^{o}; BD2: 12.191400^{o}, 109.318367^{o}; BD3: 12.189667^{o}, 109.314433^{o}), Nha Trang Sea (East Sea - Vietnam), Khanh Hoa province, Vietnam. This approach ensures comprehensive coverage of each area, providing representative data for further analysis. At each sampling station, surface water samples (collected 15 cm below the surface) were pooled into a 10-liter container and then transferred into 1-liter sterile plastic bottles. Sediment samples (200–300 g) were collected from a depth of 5 -10 cm below the surface and placed into sterile plastic bags. All samples were stored in ice boxes during transport to the laboratory for further analysis.

Bacterial isolation and identification

In this study, key waterborne and foodborne pathogens associated with humans and aquatic animals, including members of the Enterobacteriaceae family and the *Aeromonas* genus, were isolated using MacConkey Agar, *Aeromonas* Isolation Agar, and Marine Agar. For water samples, 100 mL of each sample was filtered through a 0.2 µm pore-size cellulose acetate membrane filter (Sartorius Biotech, France) to capture bacterial cells. The membranes were then placed directly onto the culture media for incubation. For sediment samples, 200 mg of each sample was suspended in 5 mL of distilled water supplemented with 3.0% NaCl (g/L) to simulate seawater conditions. A 150 µL aliquot of the diluted sediment suspension was spread onto the same set of culture media and incubated under identical conditions. All culture plates were incubated at 35 ^{o}C for 2–3 days to allow bacterial growth. Colonies were selected based on morphological characteristics and identified to the species level using the MALDI Biotyper® Sirius One IVD System with the manufacturer's IVD kit (Bruker Daltonics, Germany). Identified bacterial isolates were classified as potential pathogens according to the Risk Group Database (https://my.absa.org/Riskgroups).

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller–Hinton Agar plates, following the Clinical and Laboratory Standards Institute (CLSI) M100, 35th edition guidelines. Antibiotic selection varied by bacterial group: Enterobacteriaceae strains were tested against six antibiotic classes, including penicillins, carbapenems, fluoroquinolones, and monobactams. *Pseudomonas* strains were evaluated using cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, and monobactams. *Vibrio* strains were tested against carbapenems, cephalosporins, fluoroquinolones, and penicillins. A multidrug-resistant (MDR) phenotype was defined as resistance to at least three antibiotics from different classes. *Escherichia coli* ATCC 25922 was used as a standard reference strain in all experiments to ensure quality control.

Environmental DNA extraction

For water samples, 2 liters were filtered through a 0.2 µm pore-size cellulose acetate membrane filter (Sartorius Biotech, France) to capture bacterial cells. The membranes were subsequently cut into small pieces, and DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen, Germany) following the manufacturer's protocol. For sediment samples, 200 mg was directly processed for DNA extraction using the same kit. The extracted DNA quality and concentration were evaluated through agarose gel electrophoresis, and its

concentration was quantified using a NanoDrop™ 2000/2000c Spectrophotometer (Thermo Scientific, USA).

Quantification of 16S rRNA gene, target MGE, and ARGs

The abundance of 16S rRNA gene (bacterial density), *int*I1 (Class 1 integron integrase gene), *sul*1 and *sul*2 (sulfonamides resistance), *tet*Q and *tet*M (tetracyclines resistance) were quantified using real-time PCR with a FastGene 2x IC Green qPCR Universal Mix on the QuantStudio 5 system (Thermo Scientific, USA). The concentration of the calibration curves for each gene demonstrated efficiencies between 90% and 100%, with reliable correlation coefficients (R^2) ranging from 0.97 to 0.99. The melting temperature (Tm) of the amplified products was also assessed. The copy numbers of the target genes were calculated based on these calibration curves and expressed as copies/mL for water samples and copies/g for sediment samples.

Data-analysis

The Multiple Antibiotic Resistance (MAR) index for drug-resistant bacteria was calculated using the formula: MAR index = (number of antibiotics to which the bacteria are resistant)/(total number of antibiotics tested). A MAR index ≥ 0.2 was classified as indicative of a high risk for antibiotic resistance. The copy numbers of *int*I1, *sul*1, *sul*2, *tet*Q, and *tet*M genes were compared to the copy number of the 16S rRNA gene to assess relative abundance. Data processing and statistical analyses, including Shapiro-Wilk, Kruskal-Wallis, and Dunn's tests, were performed using RStudio.

RESULTS

Identification of potential pathogenic bacteria

A total of 27 bacterial strains were successfully isolated from sediment and water samples collected at Nha Phu (n = 12) and Bich Dam (n = 15) (Table 1). These strains were identified as belonging to 23 distinct bacterial species, including 12 Gram-positive and 11 Gram-negative species. Of these, 13 strains (48.1%) were classified as potential human and animal pathogens (risk group 2), such as *E. coli*, *Enterobacter hormaechei*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Vibrio alginolyticus* (Gram-negative), as well as *Bacillus cereus* and *Micrococcus luteus* (Gram-positive). The potential pathogens were commonly shared between Nha Phu and Bich Dam, with the exceptions of *P. mirabilis* and *P. aeruginosa*, which were detected only in Nha Phu, and *B. cereus*, which was unique to Bich Dam. Notably, the proportion of potential pathogens was higher in water samples (8/13, 61.5%) compared to sediment samples (5/13, 38.5%). These 13 potential pathogens were subsequently selected for antibiotic susceptibility testing.

Phenotypic antibiotic-resistant profile of potential pathogenic bacteria

The antibiotic resistance profiles of the Gram-negative strains varied significantly. One *E. coli* strain exhibited a multi-drug resistant phenotype, while the other remained sensitive to all antibiotics tested (Fig. 1A). *P. mirabilis* also displayed resistance to multiple drugs. Among *E. hormaechei* strains, one showed intermediate resistance to cefepime, while the other was resistant to both ceftazidime and aztreonam (Fig. 1A). Of the three *V. alginolyticus* strains, one was resistant to three antibiotics, another showed intermediate resistance to ciprofloxacin, and the third was fully susceptible to all antibiotics tested (Fig. 1B). Both *P. aeruginosa* strains shared an identical multi-drug resistant profile, showing resistance to ticarcillin, ceftazidime, and nalidixic acid (Fig. 1C). For the Gram-positive bacteria, both *B. cereus* and *M. luteus* demonstrated multidrug resistance, displaying resistance across a wide range of antibiotics, including beta-lactams, macrolides, aminoglycosides, fluoroquinolones, and glycopeptides (Fig. 1D).

Table 1. Marine bacteria and their risk group classification in the Nha Trang Sea, East Sea, Vietnam

	Surface water sample			Sediment samples		
Geographic area	Bacterial species	Gram group	Risk group	Bacterial species	Gram group	Risk group
Nha Phu	*Proteus mirabilis* NP_W_MA.1	Negative	2	*Vibrio alginolyticus* NP_S_MA.4	Negative	2
	Escherichia coli NP_W_Mac.1	Negative	2	*Pseudomonas aeruginosa* NP_S_TCBS.5	Negative	2
	Enterobacter hormaechei NP_W_Mac.4	Negative	2	*Micrococcus luteus* NP_S_Mac.1	Positive	2
	Pseudomonas aeruginosa NP_W_TCBS.3	Negative	2	*Corynebacterium sanguinis* NP_S_MA.2	Positive	1
	Bacillus subtilis NP_W_TCBS.2	Positive	1	*Rossellomorea vietnamensis* NP_S_MA.3	Positive	1
				Bacillus subtilis NP_S_TCBS.2	Positive	1
				Priestia megaterium NP_S_TCBS.4	Positive	1
Bich Dam	*Enterobacter hormaechei* BD_W_Mac.2	Negative	2	*Staphylococcus hominis* BD_W_TCBS.2.1	Positive	1
	Escherichia coli BD_W_Mac.5	Negative	2	*Vibrio alginolyticus* BD_S_MA.2	Negative	2
	Acinetobacter schindleri BD_W_TCBS.2.2	Negative	1	*Bacillus cereus* BD_S_TCBS.3	Positive	2
	Vibrio alginolyticus BD_W_MA.1	Negative	2	*Bacillus altitudinis* BD_S_MA.3	Positive	1
	Micrococcus luteus BD_W_TCBS.1.1	Positive	2	*Staphylococcus cohnii* BD_S_Mac.1	Positive	1
	Bacillus subtilis BD_W_Mac.1	Positive	1	*Bacillus subtilis* BD_S_TCBS.4	Positive	1
	Staphylococcus capitis BD_W_Mac.3	Positive	1	*Bacillus mojavensis* BD_S_TCBS.5	Positive	1
	Staphylococcus ureilyticus BD_W_TCBS.1.2	Positive	1			

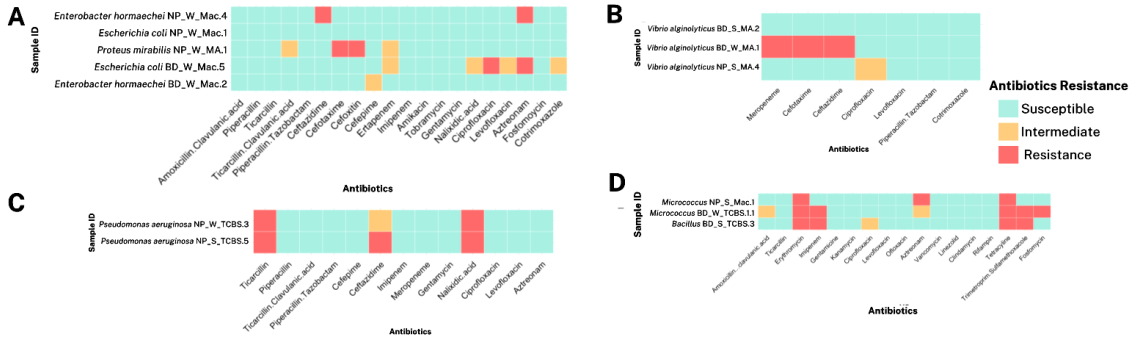

Figure 1. Antibiotic-resistant profile of Enterobacteriaceae species (A), *Vibrio alginolyticus* (B), *Pseudomonas aeruginosa* (C), *Bacillus cereus* and *Micrococcus luteus* (D)

B. cereus, *P. aeruginosa*, *M. luteus*, and *V. alginolyticus* exhibited MAR indices exceeding 0.2 (Fig. 2A). In general, bacteria isolated from water samples exhibited higher MAR indices than those isolated from sediment. However, when considering all isolates collectively, sediment-derived strains demonstrated a higher overall MAR index (0.25) compared to their water-derived counterparts (0.13) (Fig. 2B). Furthermore, the MAR index was notably higher at Bich Dam (0.2) than at Nha Phu (0.08) (Fig. 2B).

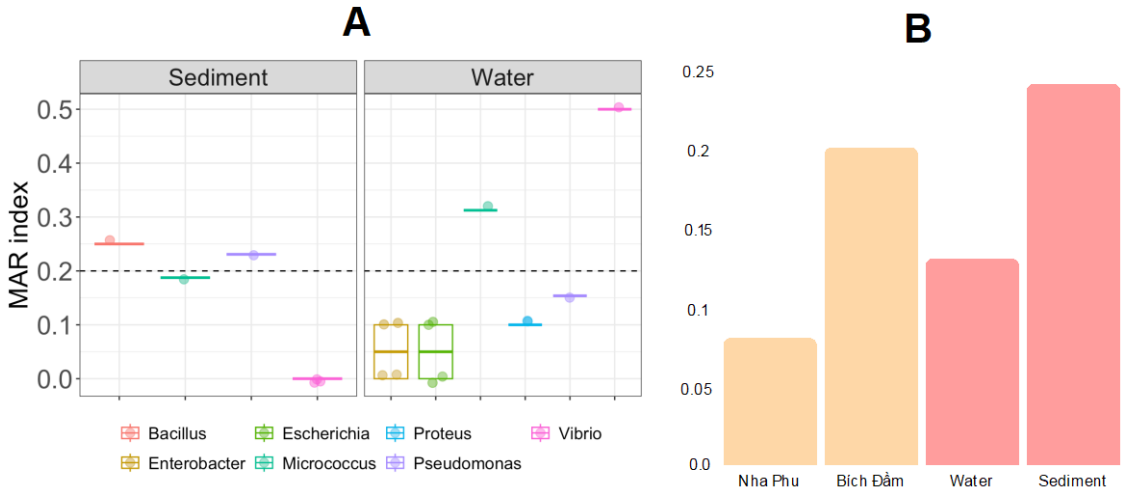

Figure 2. MAR index represented the proportion of multiple antibiotic-resistant bacteria of different groups of bacteria (A), between the sampling location and the type of samples (B)

Abundance and distribution of 16S rRNA, *int*I1, and ARGs

The absolute abundance of marine bacteria in surface water samples ranged from 3.8×10^8 to 1.5×10^9 copies/mL, while in sediment samples, it varied from 9.9×10^7 to 2.6×10^9 copies/g (Table 2). No significant difference in bacterial abundance was observed between the Nha Phu and Bich Dam areas.

Regarding ARGs, *sul*1 and *sul*2, which are associated with sulfonamide resistance, were detected in all samples (100%) (Table 2). The absolute abundance of *sul*1 ranged from 2.3×10^0 to 3.1×10^1 copies/mL in water samples and from 2.0×10^3 to 1.3×10^4 copies/g in sediment samples. For *sul*2, the abundance ranged from 2.2×10^2 to 4.5×10^3 copies/mL in water samples and from 4.8×10^4 to 4.7×10^5 copies/g in sediments.

Among tetracycline resistance genes, *tet*M was detected at all sampling locations (100%), whereas *tet*Q was found in only 60% of the locations, predominantly in the Bich Dam area (Table 2). The absolute abundance of *tet*M ranged from 0.3×10^0 to 1.7×10^2 copies/mL in water samples and from 9.0×10^1 to 2.3×10^4 copies/g in sediment samples. In *tet*Q-positive samples, the abundance varied from 2.3×10^0 to 6.0×10^1 copies/mL in water and from 2.4×10^0 to 1.1×10^5 copies/g in sediments (Table 2).

Finally, *int*I1, a gene linked to integrons, was detected in nearly all sample types, except for a single surface water sample from the Nha Phu area. Its abundance ranged from 6.2×10^2 to 2.6×10^3 copies/mL in water samples and from 4.8×10^4 to 8.2×10^5 copies/g in sediment samples (Table 2).

Table 2. Abundance and distribution of 16S rRNA, *intI*1, and ARGs across water and sediment environments in the Nha Trang Sea, East Sea, Vietnam

Geographic area	Type of sample	Sample ID	Gene copy number (copies/mL or copies/g)					
			16S rRNA	*intI*1	*sul*1	*sul*2	*tet*M	*tet*Q
Bich Dam	Sediment	BD1S	2.6×10^9	1.0×10^6	6.9×10^3	4.7×10^5	2.3×10^4	ND
		BD2S	4.6×10^8	1.6×10^5	7.2×10^3	4.6×10^5	1.5×10^2	ND
		BD3S	9.9×10^7	4.8×10^4	2.0×10^3	4.8×10^4	9.0×10^1	2.3×10^3
	Surface water	BD1W	5.2×10^8	1.2×10^2	2.3×10^0	2.5×10^2	1.7×10^2	2.4×10^0
		BD2W	3.8×10^8	5.0×10^2	6.6×10^0	1.1×10^3	1.6×10^0	2.3×10^0
		BD3W	9.7×10^8	2.2×10^2	4.6×10^0	2.2×10^2	0.6×10^0	6.0×10^1
Nha Phu	Sediment	NP1S	1.4×10^9	8.2×10^5	1.3×10^4	1.3×10^5	8.3×10^2	1.1×10^5
		NP2S	3.9×10^8	1.8×10^5	8.1×10^3	1.2×10^5	9.2×10^2	8.8×10^2
		NP3S	1.3×10^9	1.7×10^5	4.1×10^3	1.7×10^5	1.0×10^3	ND
	Surface water	NP1W	9.2×10^8	6.2×10^2	3.1×10^1	3.3×10^3	0.8×10^0	ND
		NP2W	1.5×10^9	2.6×10^3	3.0×10^1	4.5×10^3	0.9×10^0	1.6×10^1
		NP3W	7.6×10^8	ND	1.4×10^1	7.2×10^2	0.3×10^0	ND

Note: ND: not detected.

Overall, the relative abundances of ARGs and the *int*I1 gene, normalized to the 16S rRNA gene, were higher in the Nha Phu area compared to Bich Dam (Fig. 3A). However, no significant differences were observed between sample types (water and sediments) or between the Nha Phu and Bich Dam areas.

The mean absolute abundance of the *sul*1 gene was significantly higher in sediment samples than in surface water samples (p = 0.004), with no significant difference between the Nha Phu and Bich Dam areas (p = 0.4) (Fig. 3B). Similarly, *sul*2 abundance was significantly higher in sediments than in surface water (p = 0.01), while no significant difference was observed between the two areas (p = 0.09).

No significant differences in *tet*Q abundance were found between surface water and sediment samples (p = 0.7) or between the Nha Phu and Bich Dam areas (p = 0.6) (Fig. 3B). Although *tet*M abundance did not differ significantly between water and sediment samples (p = 0.6), it was significantly higher in Bich Dam than in Nha Phu (p = 0.01). In contrast, *int*I1 abundance was significantly higher in sediments than in water samples (p = 0.02) but did not differ between the two sampling areas (p = 0.4) (Fig. 3B).

The PCoA plot revealed a distinct separation between the two sampling areas, with types of environment (water vs. sediment) emerging as the more influential

factor (Fig. 3C). A pronounced separation based on the types of environment was primarily observed along the PCo2 axis (34.7%). Sediment samples clustered predominantly in the lower half of the plot, while water samples were mainly positioned in the upper half, indicating that the types of environment had a greater impact on the distribution of ARGs and *int*I1 than geographical location.

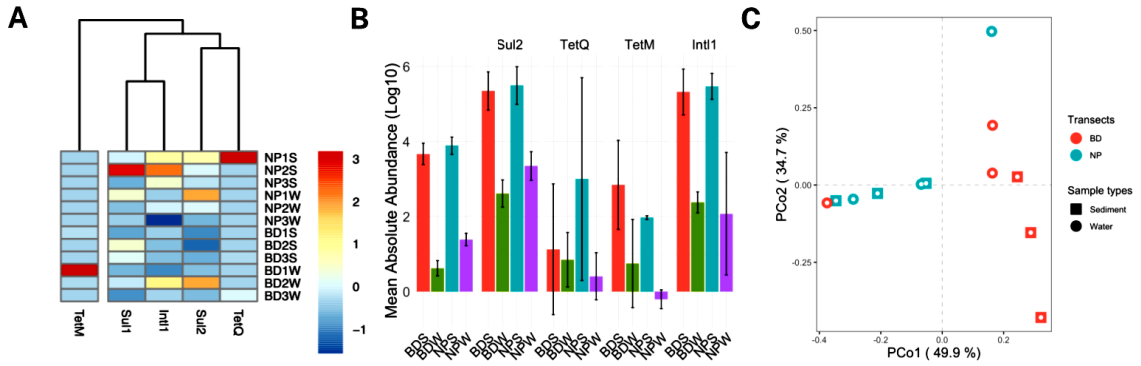

Figure 3. Abundance and distribution of ARGs and *int*I1 gene across types of environments (water sv sediment) and geographic areas: Heatmap of log10 of relative abundance of ARGs and *int*I1 gene normalized to the 16S rRNA gene (A); Clustered bar plot of mean absolute abundance (log10) of ARGs and *int*I1 gene (B); PCoA of the absolute abundance of ARGs between different types of environment and geographical areas (C)

DISCUSSION

Although ARB has existed in nature long before the advent of antibiotics, the extensive use of these drugs in healthcare, agriculture, and aquaculture has significantly accelerated the evolution and dissemination of ARB and ARGs across various environments (Kim &Cha, 2021; Xu et al., 2023; Yin et al., 2023). While substantial research has focused on the prevalence and transmission of ARB and ARGs in clinical and agricultural settings, their occurrence and dynamics in environmental compartments remain relatively complicated. Moreover, most studies on AMR circulation in natural environments have primarily focused on inland ecosystems (Ajulo & Awosile, 2024), with marine environments receiving comparatively less attention. Among marine ecosystems, coastal areas serve as transitional zones between terrestrial and marine environments and are heavily impacted by various human activities (Adenaya et al., 2024; Fruergaard et al., 2023; Schar et al., 2021). As a result, they represent important reservoirs for the emergence and transmission of ARB and ARGs within communities. This study examines the prevalence of ARB and ARGs associated with tetracycline and sulfonamide, alongside the mobile genetic-element gene *intI1*, a key driver of ARG mobilization and dissemination. Conducted in Nha Trang, one of Vietnam's most dynamic coastal regions, the research aims to advance understanding of resistance patterns and underlying mechanisms in complex aquatic environments.

In our study, potential pathogenic species exhibited MDR phenotypes including *E. coli*, *P. mirabilis*, *P. aeruginosa*, *V. alginolyticus*, *B. cereus* and *M. luteus* in both sediment and water samples underscores the potential health risks associated with the sea environment in Nha Trang. A previous study also found antibiotic-resistant Gram-positive and Gram-negative bacteria isolated at Hon Mot in the Nha Trang Bay (Pham et al., 2018). A subsequent study isolated 109 pathogenic bacteria including *Vibrio* spp., *Salmonella* spp., *Shigella* spp., and *Aeromonas* spp. from

water and sediment of aquaculture farms in Dam Bay and Hon Mieu of the Nha Trang Bay (Nguyen-Kim et al., 2020). Notably, these bacteria were highly resistant to tetracycline (96.6%) and Nifuroxazide (92.5%). Overall, the prevalence of ARB ranged from 33.3% to 68.9% %. Unfortunately, no data related to ARGs was reported in these two studies. Our study revealed that both *sul*1 and *sul*2, conferring sulfonamide resistance, were ubiquitous, detected in all samples, highlighting the widespread presence of this resistance mechanism. While *tet*M also showed high prevalence, *tet*Q was detected less frequently, suggesting potential differences in the selective pressures driving tetracycline resistance. A recent study investigated the occurrence and distribution of antibiotic-resistant Enterobacteriaceae in water and sediment samples from Nha Trang Bay, and identified more than 57% of them exhibited MDR phenotypes, with *E. coli*, *K. pneumoniae*, and *C. freundii* being the most prevalent species (Tran et al., 2025). Additionally, the authors reported for the first time the abundance of ARGs including *sul*1, *sul*2, *sul*3, *tet*Q, *tet*B, *mec*A, *bla*VIM, and *bla*KPC in marine environments surrounding the Nha Trang city. These findings highlight the need for further monitoring and strategies to mitigate the spread of AMR in Vietnam's coastal environments.

The 16S rRNA gene copy numbers observed across all samples, ranging from 3.82×10^8 to 2.63×10^9 copies/mL, highlight the high abundance of the marine microbiome, including potential pathogens. This increased microbial presence poses a heightened risk of infection and disease for humans and animals, as well as potential threats to the food chain and food safety. For instance, previous study reported a very high prevalence of *E. coli* carrying β-Lactamase-encoding genes of CTX-M-1 (50.7%), CTX-M-9 (41.5%), TEM (59.9%), and SHV (2.8%) groups in retail meats and shrimp collected in markets of Nha Trang City. In addition, the author also detected 85.9% of ESBL-producing *E. coli* with MDR phenotypes (Le et al., 2015). Seriously, mobile *mcr* genes associated with colistin resistance were found in fish and shrimp collected from markets in Nha Trang. These findings highlight the significant risk of the spread of MDR bacteria in the community in this city (Le et al., 2021).

It has been well demonstrated that integrons playd important roles in multiplying and facilitating the transmission of ARGs in bacteria via horizontal gene transfers (Bhat et al., 2023; Tokuda & Shintani, 2024). Our study found a wide spread of the *int*I1 gene in both water (87%) and sediment (100%) samples of the Nha Trang Sea, particularly with a high abundance in sediments ranging from 4.8×10^4 to 1.0×10^6 copies/g, which are comparable with data obtained at hotspot areas of AMR from global studies. This evidence underlines a robust potential for ARGs transfer in this region and highlights the need to monitor ARGs in aquatic ecosystems. Furthermore, a recent study observed significant correlations between *sul*3, *tet*B, *bla*VIM, *bla*KPC, and *int*I1 with bacterial density, chlorophyll A, phosphorus, and temperature (Tran et al., 2025). In agreement with this finding, our study highlights the high risk of transmission and spread of MDR bacteria and ARGs in the whole coastal areas in the Nha Trang Sea. Together, these data will serve as a valuable reference for comparison with our recent study, allowing us to assess trends and changes in AMR in this region over time.

CONCLUSION

This study identifies the presence of potential multidrug-resistant pathogens in the marine environments of Nha Trang City. It also reveals a significant abundance of ARGs associated with sulfonamides and tetracyclines. Additionally, the high prevalence of the integron-integrase gene *int*I1 in both water and sediment underscores the importance of further research to pinpoint specific sources of contamination, understand

the mechanisms behind ARG spread, and assess the potential risks to human and animal health.

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