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# Occurrence and driving mechanism of antibiotic resistance genes in marine recreational water around Qinhuangdao, China

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Rapid urbanization and other human activities have exacerbated the global human health risks associated with antibiotic resistance genes (ARGs). Swimming in contaminated waters is one of important pathways by which humans can be exposed to ARGs. However, little is currently known about the overall levels of ARGs in recreational waters and the forces driving their occurrence. In this study, we analyzed the ARGs and bacterial communities in water samples taken from five recreational coastal waters (RCWs) and their adjacent estuaries in the Qinhuangdao area of Bohai Bay, China, using high-throughput quantitative PCR and 16S rRNA gene amplicon sequencing technique. The relative abundances of ARGs in RCWs varied greatly, and occurred at levels close to, or even higher than, those in the neighboring estuaries, ranging from  $9.4 \times 10^{-2}$  to 2.5 copies/16S rRNA gene. A total of 159 different ARGs able to express resistance to almost all antibiotics were detected in all of the water samples.  $\beta$ -lactamase resistance genes (*bla*TEM gene) were the most abundance of all ARGs. Patterns of ARG occurrence were significantly correlated with those of bacterial communities (Mantel test,  $P < 0.05$ ). A partial redundancy analysis showed that differences in bacterial communities accounted for 50.3% of the total ARG variations, which was much higher than the sum of the contribution of mobile genetic elements (18.1%) and the other selected environmental factors (8.3%). Principal coordinate analysis based on Bray-Curtis distance revealed the similarities between ARG profiles in the RCWs and in their adjacent estuaries. To our knowledge, this is the first study to report the ARG profiles in RCWs using high throughput qPCR technology. These results implied that it is necessary to develop proper regulations and monitoring strategies of RCWs for reducing risks of ARGs on human health.

## KEYWORDS

antibiotic resistance genes (ARGs), mobile genetic elements (MGEs), bacterial community, recreational coastal water, human public health

## Introduction

The overuse of antibiotics has accelerated the emergence and spread of antibiotic resistance genes (ARGs) in the environment (Wen et al., 2015). ARGs have been found in a wide variety of environments including agricultural soils (Sun et al., 2020), urban wastewater treatment plants (Freeman et al., 2018), lakes (Y. Q. Wang et al., 2021), rivers (Pruden et al., 2006) and aquacultural facilities (P. Gao et al., 2012). Microorganisms in the environment can contribute to, or acquire, ARGs from human pathogenic bacteria, as demonstrated by the significant similarities in their sequences (Tomova et al., 2015; Jing Yang et al., 2013). Increased levels of ARGs in the environment have led to growing concerns about an impending global public health crisis (Pruden et al., 2006). It has been estimated that the number of deaths arising from antibiotic resistant pathogens may exceed 10 million by the end of 2050 (O'Neil, 2014), and that ARGs will surpass cancer as the most serious risk factor for human health. As emerging contaminants (A. Pruden et al., 2006), ARGs replicate not only through bacterial growth but also can be transferred between different bacterial species mediated by mobile genetic elements (MGEs) (von Wintersdorff et al., 2016). The amount of ARGs can increase and spread if they are selected by antibiotic pollutions or are co-selected by ecologically favorable determinant present in the same MGEs (Blahova et al., 1999). Therefore, identifying environmental reservoirs of ARGs and exploring their routes of transmission are critical for the prevention of ARG related infections.

Estuaries occur at the boundary between terrestrial/freshwater and coastal ecosystems (Stewart et al., 2008; Marti et al., 2014). Approximately half of the world's population lives within 100 kilometers of the coastline (Culliton, 1998). Generally, estuaries receive mass discharges of waste from industry and other human activities (Wright et al., 2008), such as wastewater treatment plants (WWTP) (Wu et al., 2018; Bi Weihong et al., 2022), medical wastewater (Rodriguez-Mozaz et al., 2015), livestock rearing, aquaculture and agricultural wastewater (Jang et al., 2018). Traditional WWTPs are not generally effective in removing ARGs and the ARG abundance in the effluent can be as high as  $3.13 \times 10^6$  copies/mL (H. Chen & Zhang, 2013; H. W. Huang et al., 2019). Terrigenous pollutants, including heavy metals, antibiotics, ARGs and drug-resistant bacteria, are also washed into rivers and then into estuaries. Surface waters also receive wastewater and municipal sewage from nearby factories and cities. It has been shown that rivers in China generally contain various ARGs, including the Yangtze, Yellow, Haihe and Huaihe Rivers (Zhuang et al., 2021). Moreover, the specificity of the water quality environment and the increasing development of tourism can also affect the evolution of microbial resistance (Su et al., 2022). The resistant bacteria will be brought into the coastal tourist area near the

shore with the rain, tide, and currents. Swimming in recreational coastal waters (RCWs) is an important route by which humans are exposed to pollutants in the coastal environment. Many studies have reported various pollutants in RCWs, including heavy metals (Bigus et al., 2016), nitrogen, phosphorus, viruses (Wei et al., 2020), microplastics (F. L. Gao et al., 2021), and pollutants resulting in elevated chemical oxygen demands, which may facilitate the spread of ARGs (Stepanauskas et al., 2006; Tandukar et al., 2013; Forsberg et al., 2014). However, the overall profiles, influent factors, and driving mechanisms of ARGs in RCWs are still not fully understood.

The dynamics of ARG occurrence are far more complex than the selective pressure of antibiotics (Tamminen et al., 2011). A growing number of studies have reported that shifts in bacterial communities are the main driver of ARG accumulation in the environment (Zhang et al., 1800; Su et al., 2015). Horizontal gene transfer (HGT) mediated by MGEs is also an important mechanism of ARG propagation (K. Zhang et al., 2021). Environmental microorganism, particularly pathogenic bacteria, can acquire ARGs from different species of bacteria via HGT to express antibiotic resistant and even become the "superbugs" endangering public health (von Wintersdorff et al., 2016). Environmental factors, such as nitrogen and phosphorus concentrations (Ali et al., 2016), pH (H. N. Huang et al., 2017), salinity (Y.-J. Zhang et al., 2019), and temperature (J. Yang et al., 2020), may also be predominant factors governing the distribution patterns of environmental ARGs. Therefore, it is necessary to explore the overall ARG profiles in RCWs as well as all of the possible factors affecting their occurrence to discover the predominant drivers and to control their propagation and thus reduce potential health risks.

The Bohai Sea has a coastline of about 3,800 kilometers and is fed by large number of rivers. Qinhuangdao, located in the north part of the Bohai Sea, is one of the most important tourist cities in northern China, and is greatly impacted by human activities (L. H. Zhu et al., 2014). The "Qinhuangdao Recreational Coastal Water Management Regulations" stipulate the minimum straight-line distance between RCWs and the nearest estuary, fishing port, tourist wharf, fishing boat berth and shallow sea aquacultural area to avoid the spread of pollutants (Qinhuangdao Municipal People's Government, 2019). However, it is unclear whether the above provisions can prevent the ARG impacts of estuaries on RCWs. In this study, water samples were collected from the estuaries of typical rivers and their adjacent RCWs around Qinhuangdao: (1) to investigate the ARG profiles and bacterial communities in estuaries and their RCWs; (2) to determine the influencing factors and driving mechanisms of ARG occurrence in estuaries and their adjacent RCWs; and (3) to explore the potential antibiotic resistance risks to human health under current regulations.

## Methods and materials

### Sample collection, water chemistry and DNA extraction

Water samples were collected from the estuaries and their adjacent RCWs around Qinhuangdao City, located on the north coast of Bohai Bay, China. Detailed descriptions of the sampling sites are shown in [Figure S1](#). Water samples (~5L) were taken during December 2020 on three consecutive days without rainfalls during the preceding week. All water samples were stored on ice in sterile containers and brought back to the laboratory within six hours for further treatment.

The bacteria cells in each water sample were collected by filtering through 0.22 mm mixed cellulose esters filters (Millipore, USA) and stored at -20°C for subsequent total extraction of genomic DNA using a QIAGEN Extraction Kit (Qiagen Instruments, USA), following the manufacturer's instructions. The purity and integrity of the extracted DNA were assessed using a Nano Drop spectrophotometer (Thermo Fisher Scientific, USA) and agar gel electrophoresis, respectively. The extracted DNA was stored at -20°C. Temperature, pH and salinity were measured in situ using a multiparameter water quality analyzer (Hydrolab DS5, Hach Company, Loveland, USA). Ammonia-nitrogen (NH<sub>4</sub><sup>+</sup>-N), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N) ([Rider and Mellon, 1946](#)), nitrite nitrogen (NO<sub>2</sub><sup>-</sup>-N) ([J. Yang et al., 2020](#)), and phosphate (PO<sub>4</sub>-P) were measured according to standard methods ([B. Wu et al., 2014](#)).

### High-throughput quantitative PCR of ARGs

HT-qPCR was performed using a Wafergen SmartChip Real-time PCR System (Anhui Microanaly Gene Technologies Co., Ltd, China) to detect and quantify ARGs, as described previously ([Ouyang et al., 2015](#)). Quality assurance/quality control was conducted following the standard protocols provided by Wafergen Biosystems. A total of 296 primer sets were used targeting at 285 ARGs, eight transposases, three integrons and one 16S rRNA gene. Detailed information on the primers is listed in [Table S1](#). The 285 ARGs could express antibiotic resistance to almost all antibiotics by 4 mechanisms. HT-qPCR assay was performed in a 100 nL reaction system consisting of a 1 × LightCycler 480 SYBR Green I Master mix, 0.1 mg/mL albumin from bovine serum, 500 nM of each primer and 2 ng/μL DNA template. A non-template control was included on each chip for each primer set. The qPCR reaction mixture was first added to a microarray using nanoscale multi-sample point sampling instrument, and then qPCR reaction was performed using a cycler with the thermal cycles: 95°C for

10 min, 40 cycles of 95°C for 30 s, and 60°C for 30 s, ending with a melting curve analysis auto-generated by the Wafergen program.

The HT-qPCR results were analyzed using SmartChip qPCR (v 2.7.0.1) software to remove the results with multi-peak melting curve and with amplification efficiencies beyond the range of 1.8-2.2. A threshold cycle (CT) value of 31 was used as the detection limit. All qPCR reactions were conducted in triplicate, and if more than two were amplified, the result was regarded as positive. Relative copy numbers of ARGs was calculated according to the following equation:

$$\text{Gene copy number} = 10^{-(31-CT)/(10/3)}$$

Relative ARG copy number = ARG copy number/16S rRNA gene copy number

Where, CT is the cycle threshold.

The result of the primer sets targeting at the same gene were combined. For example, blaOXA10-01 and blaOXA10-02 both target at the blaOXA10 gene according to their nomenclature ([Jacoby, 2006](#)).

### Bacterial 16S rRNA gene sequencing and data processing

Bacterial communities were investigated using a 16S rRNA gene amplicon sequencing technology (Anhui Microanaly Gene Technologies Co., Limited, China). The V3-V4 region of the 16S rRNA gene was amplified using barcodes and the primer sets 341F (5'-CCTACGGGNGGCWGCAG-3') and 765R (5'-GACTACNVGGGTATCTAAT-3'). The genomic DNA was used as templates to conduct PCR using Taq DNA Polymerase (Vazyme, Nanjing, China). PCR products were purified using a QIAquick Gel Extraction Kit (QIAGEN, USA) and pooled in equimolar for sequencing using an Illumina MiSeq PE300 platform. Paired-end sequences were joined after the barcode and primers were removed using FLASH ([Magoc and Salzberg, 2011](#)). FLASH software was also used to remove short reads, chimeras and ambiguous N ([Magoc and Salzberg, 2011](#)). The sequences were clustered into Operational Taxonomic Units (OTUs) with a threshold of 97% similarity using the VSEARCH method ([Rognes et al., 2016](#)). Representative sequences were selected for further OTU annotation using a default method involving assignment to the Greengenes Database taxonomy ([Wang et al., 2007](#)). Species diversity and α-diversity of the bacterial communities were estimated with the observed species, Chao1, Shannon, Simpson and PD\_whole\_tree index, which were calculated using a script in QIIME software. Bacterial communities in different groups (β-diversity) were compared using the unweighted pair group method with arithmetic mean (UPGMA), principal coordinate analysis (PCoA) based on Bray-Curtis distances, and the Adonis test.

## Network analysis

The co-occurrence patterns among ARGs, MGEs and bacterial communities were revealed by network analysis using Cytoscape (3.8.2) with the CoNet plug-in (Sarkar and Chang, 1997). Pairwise correlations were calculated by the Mutual information, Spearman's correlation, Pearson's correlation, Bray-Curtis dissimilarity and Kullback-Leibler dissimilarity tests. Correlations with a P value above 0.9 and a significance level below 0.05 were considered significant. ARGs or bacterial taxa detected in less than three samples were discarded in correlation calculations. Correlation efficiencies and P values were analyzed using the Simes method implemented with the CoNet plug-in to avoid false-positive correlations (Sarkar and Chang, 1997). The network topology was visualized in Gephi (0.9.2) with the Frucherman Reingold algorithm (Dalcin and Jackson, 2018).

## Statistical analyses

Averages and standard deviations of all data were determined using Microsoft Excel 2010 (Microsoft Office 2010, Microsoft, U.S.A.). The normalized ARG abundances were plotted with OriginPro 2017 (OriginLab, U.S.A.). Heat maps analyzing the ARGs data of water samples were generated with using the Pheatmap package in R (Version 4.1.2). Mantel test, PcoAs based on Bray-Curtis distance, Redundancy analyses (RDA) and partial RDAs were performed using R4.1.2 with the vegan package. Aggregated boosted tree (ABT) analysis was performed using R2.7.0 with the gbmplus package. Significant differences between estuaries and RCWs were tested using the Student–Newman–Keuls (S–N–K) test (SPSS V20, IBM, U.S.A.). Pearson's correlation coefficients among ARG and MGE abundances were calculated using SPSS.

## Result

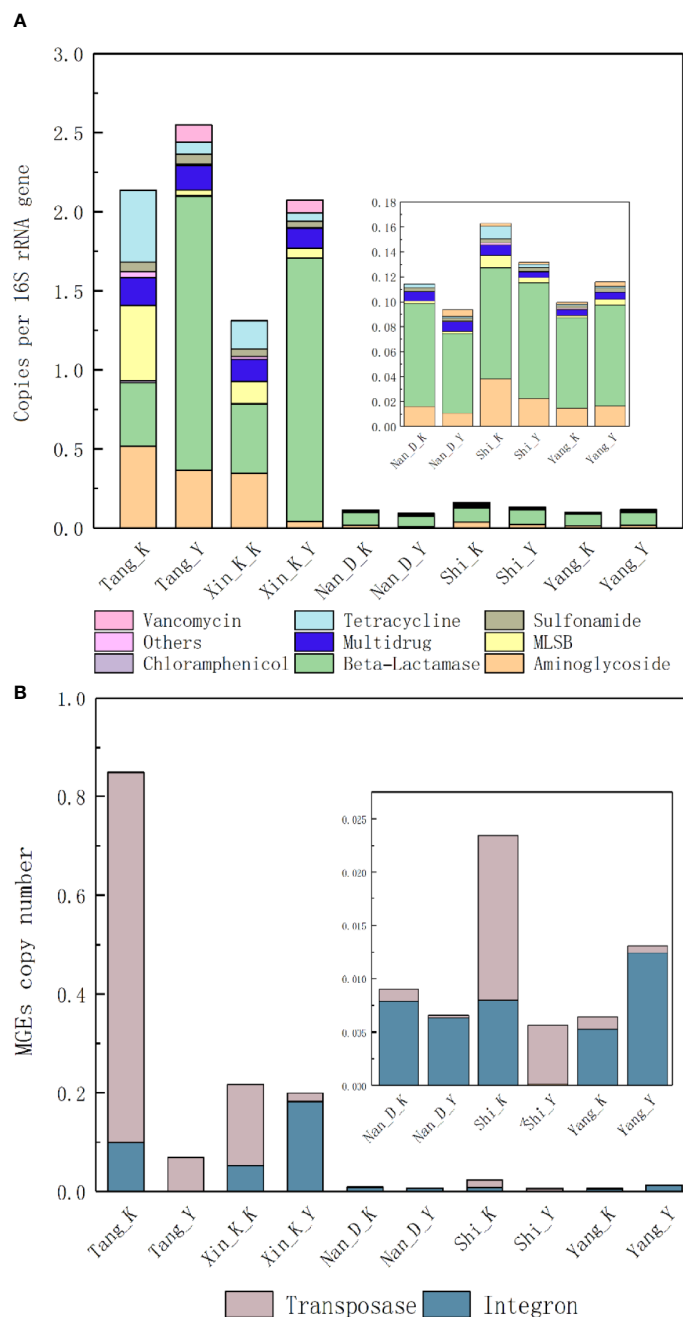
### Abundance and diversity of ARGs

A total of 159 ARGs and 5 MGEs were detected in the 10 water samples from RCWs and estuaries in Qinhuangdao, China. An average of 114 ARGs were detected in the estuaries and 94 in the RCWs. However, there were not necessarily fewer ARGs in the RCWs than in their adjacent estuaries, such as the Nandai and Yang Rivers (Figure S2). Aminoglycoside and  $\beta$ -Lactamase were the most dominant types of resistant genes, followed by Macrolide-Lincosamide-Streptogramin B (MLSB) and tetracycline resistant genes, accounted for 30.8%–83.9% of the total ARGs in each sample (Figure S2). ARGs potentially

conferring resistance to vancomycin were widely distributed among the estuaries and RCWs, with occurrence ranging from 2.34%–6.94%. It should be noted that vancomycin is critically important for human medicine and is considered the last line of treatment for bacterial infections. In the 159 ARGs, antibiotic deactivation, efflux pump, and cellular protection are the most predominant resistance mechanisms (Figure S2).

The relative abundances of ARGs in water samples from the Tang and Xinkai Rivers are significantly higher than those in other samples ( $P < 0.01$ ), as shown in Figure 1A. In contrast to the other estuaries, the most abundant type of ARG in the Tang River was aminoglycoside (17.3%). The relative abundance of ARGs in water samples from the Tang and Xinkai Rivers ranged from 1.3–2.5 copies/16S rRNA copies, about 20 times higher than those of the other samples (0.21–0.29copies/16S rRNA gene). A total of 10 MGEs were detected, including eight transposases and two integrases with relative abundances ranging from 2.7%–28.5%. As shown in Figure 1B, the relative abundance of MGEs in all RCWs and estuaries ranged from  $5.6 \times 10^{-3}$ – $8.5 \times 10^{-1}$  copies/16S rRNA genes. The normalized copy number of transposases and integrases was significantly different among sampling sites. For example, integrase accounted for 96.0% of MGEs in the RCW adjacent to the Nandai River estuary, but only transposase genes were detected in the RCW close to estuary of the Tang River (0.11copies/16S rRNA genes).

There were significant differences in the overall structure of ARGs in the different water samples (Adonis test,  $P < 0.05$ ). PCoA based on Bray-Curtis distance (Figure 2A) showed that water samples from the Nandai, Yang and Shi Rivers clustered together and separated from the other samples. The first two PCs explained a total of 68.1% of the variance in ARGs, with PC1 explaining more than half (50.5%) of the variation. Samples from different locations were generally separated along the first PC, and samples from the same estuary and its adjacent RCW were separated along PC2, which explained 17.6% of the variation, indicating the minor differences between ARG profiles of an estuary and its adjacent RCWs. Hierarchical clustering analysis further verified such variances in ARG profiles (Figure 3). The estuary samples from the Tang and Xinkai Rivers were separated from the other water samples, which clustered together. As shown in Figure 3, all of the ARGs detected could be divided into three subgroups: (A) ARGs with high relative abundance in all water samples, such as the blaTEM, folA, blaOXA10 and aadA5 genes; (B) ARGs with high relative abundance in the estuaries of Tang and Xinkai Rivers, such as the strB and aadA genes; and (C) ARGs with high relative abundance in other sampling sites, such as the VanC and mphA genes. A detailed table of ARG abundance with names can be found in the Supplementary Materials (Table S2).



**FIGURE 1** Normalized abundance of (A) ARGs and (B) MGEs in sediment of estuaries and recreational coastal waters. Normalized copy number of ARGs presented as number of ARGs per 16S rRNA genes. Multidrug resistance genes are defined as the resistance to multiple antibiotics with different structures and antimicrobial mechanisms.

### Characteristics of microbial community structure

After assembling and quality filtering, a total of 640,000 high-quality sequences were obtained from 10 samples, ranging from 45,663-95,376 sequences per sample. These sequences were

assigned to 8,675 OTUs at the 97% similarity level, with the number of OTUs in each single sample ranging from 638-1,112. Species accumulation curves and rank abundance curves indicated that the bacteria species in the estuarine samples were generally higher than those in the adjacent RCWs (Figure S3), Proteobacteria and Bacteroidetes were the



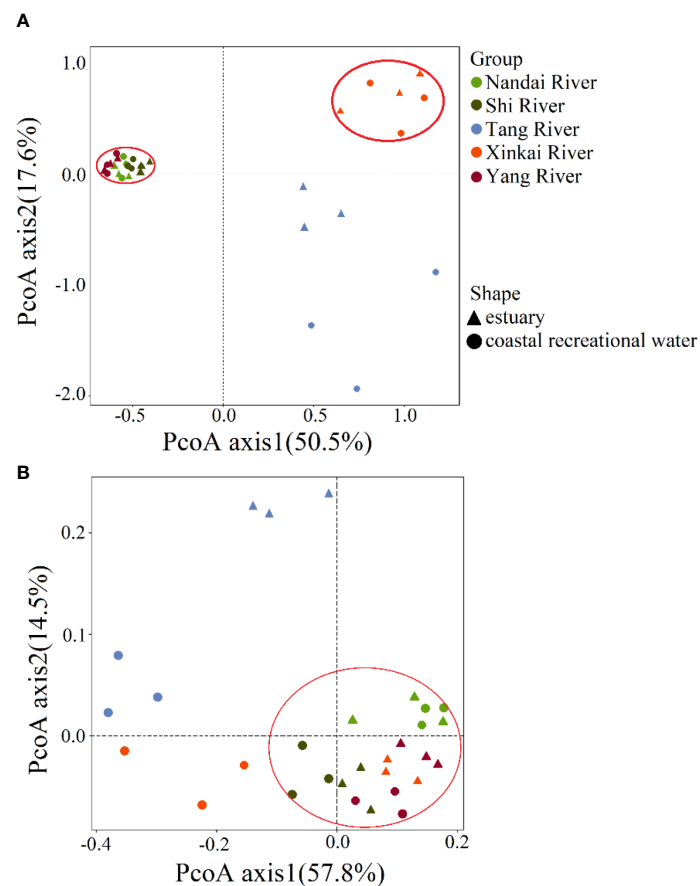


FIGURE 2

Principal coordinates analysis based on Bray-Curtis distance showing the overall distribution pattern of (A) ARGs and (B) microbial communities in water samples of estuaries and recreational coastal.

dominant phyla in all samples, accounting for 57.1–83.5% of the bacterial communities. Gammaproteobacteria and Alphaproteobacteria were the major classes of Proteobacteria, while Flavobacteriia was the major class of Bacteroidetes. The compositions of the bacterial communities at different levels are shown in Figure S4. PCoA analysis based on Bray-Curtis distance showed that estuarine bacterial communities and their corresponding RCWs were relatively similar except in the Tang and Xinkai Rivers. As shown in Figure 2B, the first two PCs explained 57.8% and 14.5% of the differences in bacterial community composition, respectively. The genus-level UPGMA hierarchical analysis confirmed that the bacterial communities in the Shi and Yang River regions were similar at the genus level (Figure S5). *Psychrobacter*, *Litoreaibacter*, *Sulfotomarina*, *Planktomarina* and *Loktanella* were the most dominant bacteria, accounting for 29.5–49.6% of the total genera. Interestingly, the bacterial communities in samples from the Shi and Yang Rivers were similar even though they are not geographically adjacent, indicating that bacterial communities in coastal environments were affected by more

complex factors than mere diffusion between. No significant differences were observed between bacterial communities from estuaries and their adjacent RCW (Anosim,  $P > 0.05$ ), but certain bacterial species were only observed in estuaries or in RCWs. For example, *Campylobacteraceae* and *Arcobacter* were prevalent in estuaries but rarely detected in RCWs. Whether they can be used as biomarkers to distinguish estuarine samples from RCWs requires further research. It is worth noticing that *Arcobacter*, a potential human pathogen, was widely distributed in estuaries. Even though its detection limit in RCWs was low in this study, its dissemination and fate in coastal environments requires further surveillance and attention.

## Correlations between ARGs, MGEs and microbial community structure

Significant correlations were detected among the all classes of ARGs (Pearson's correlation), indicating that the spread of various ARGs may share the same dissemination mechanism.

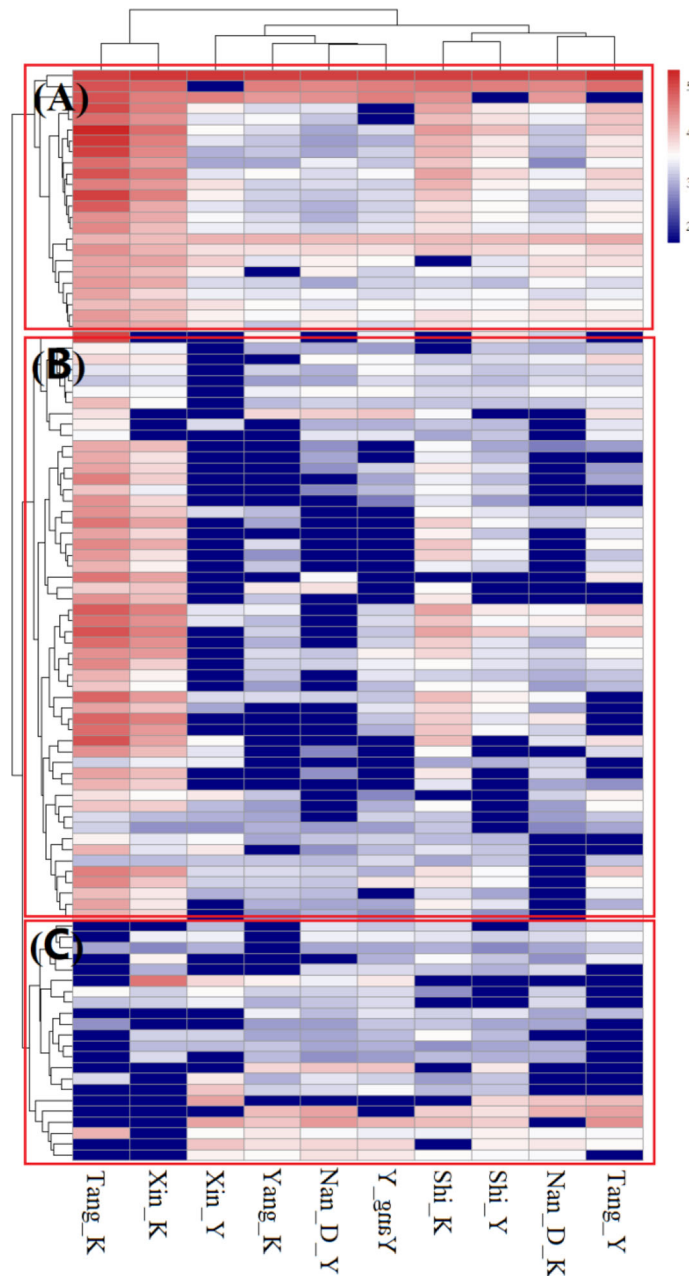


FIGURE 3

ARG profiles in sediment of estuaries and recreational coastal waters. Each column is labeled with a sample name. K represents water samples from estuaries and Y represents water samples from recreational coastal water. Plotted values are the logarithm-transformed relative abundance of each ARGs. Columns and rows were clustered based on Bray-Curtis distance. (A) ARGs with high relative abundance in all of the water samples; (B) ARGs with high relative abundance in estuaries of Tang and Xinkai Rivers; (C) ARGs with high relative abundance at the other sampling sites.

The abundance of integrons was not correlated with any particular class of ARG, but the total abundance of transposases genes were significantly correlated with almost all classes of ARG, except for  $\beta$ -Lactamase, sulfonamide, and vancomycin resistance genes, indicating that transposons played an important role in ARG propagation in coastal

environment. Several ARGs (*bla*TEM, *str*B, *bla*VEB, *sul*I genes), generally embedded in transposons, were detected with the highest normalized copy number, indicating that transposons were the most common carrier of ARGs in coastal waters. With the exception of  $\beta$ -Lactamase and vancomycin resistant genes, the normalized abundance of ARGs were

correlated with those of MGEs, with correlation coefficients ranging from 0.67-0.99 (Table 1, Person's correlation,  $P < 0.05$ ), indicating that ARGs may be readily transferred within bacterial communities. These correlations were based on the normalized abundances of ARGs and MGEs, suggesting that MGEs mediated HGT, contribute to the propagation of ARGs in coastal waters and lead to the enrichment of those ARGs in bacterial communities.

The patterns of co-occurrence among ARGs and MGEs were analyzed using co-occurrence network analysis (Figure 4) based on the strong and significant correlation between ARGs and MGEs. The network structure consists of 25 nodes and 20 edges, which could be divided into nine modules with a module degree of 0.72. The line in the Figure 4 shows a strong and significant correlation between two nodes ( $r > 0.9$ ). The most densely connected node was defined as a "hub" in the module. For example, the *aadA2* gene is the hub in its module and is significantly correlated with the *blaOXA10*, *catB3*, *qacEdelta1* and *tetM* genes. The hubs can be used as indicators for all ARGs in a module to predict dynamic changes of other ARGs in the same module. Most modules contained different types of ARGs or MGEs. For example, module IV includes the aminoglycoside resistant genes (*aadA2* gene) and the tetracycline resistance genes (*tetR* gene), and module VIII includes the *vgbB* gene and *aadD* gene, which belong to MLSB and aminoglycoside resistance genes. The correlation between transposon (*tnpA* gene) and aminoglycoside resistant gene (*aac(6')*-Ib(aka *aacA4*)) further indicated that transposons could contribute to the propagation of ARGs in coastal environments. The correlations between different ARGs suggested that they are carried by the same bacteria or located in the same MGE. We further analyzed the patterns of co-occurrence among ARGs, MGEs and bacterial communities at the genus level. We hypothesized that a strong nonrandom, significant correlation between microorganisms and ARGs ( $R^2 > 0.9$ ,  $P < 0.05$ ) could indicate the identity of the host of an ARG. For instance, *Acinetobacter* may be the host cell of an aminoglycoside gene

(*aac(6')*-Ib(aka *aacA4*)), and the  $\beta$ -lactamase resistance gene (*blaVEB*) was probably carried by the genus *Phycisphaera*.

We also studied the differences between the abundances of bacteria that directly correlated with ARGs in RCWs and estuaries. The bacteria which could be recognized as hosts of ARGs were detected with relatively high abundance in estuaries but low abundance in RCWs, and were perhaps present at levels below the limits of detection. The abundance of *Succinispira*, which is correlated with the trimethoprim resistant gene (*dfrA*) ranged from 0-  $8.3 \times 10^{-5}$  in estuaries, but was rarely detected in RCWs. *Acinetobacter* is a potential human pathogen, and was strongly and significantly associated with aminoglycoside resistant gene (*aac(6')*-Ib(aka *aacA4*)). This potentially resistant pathogen was detected in all of the water samples except for the RCWs adjacent to the Nandai River. Together, these results suggested that environmental bacteria and pathogens had the potential to evolve antibiotic resistance in coastal environments and could spread to humans swimming in these waters so posing serious risks to human health.

## Relationships among bacteria communities, mobile genetic elements, environmental factors and antibiotic resistance genes

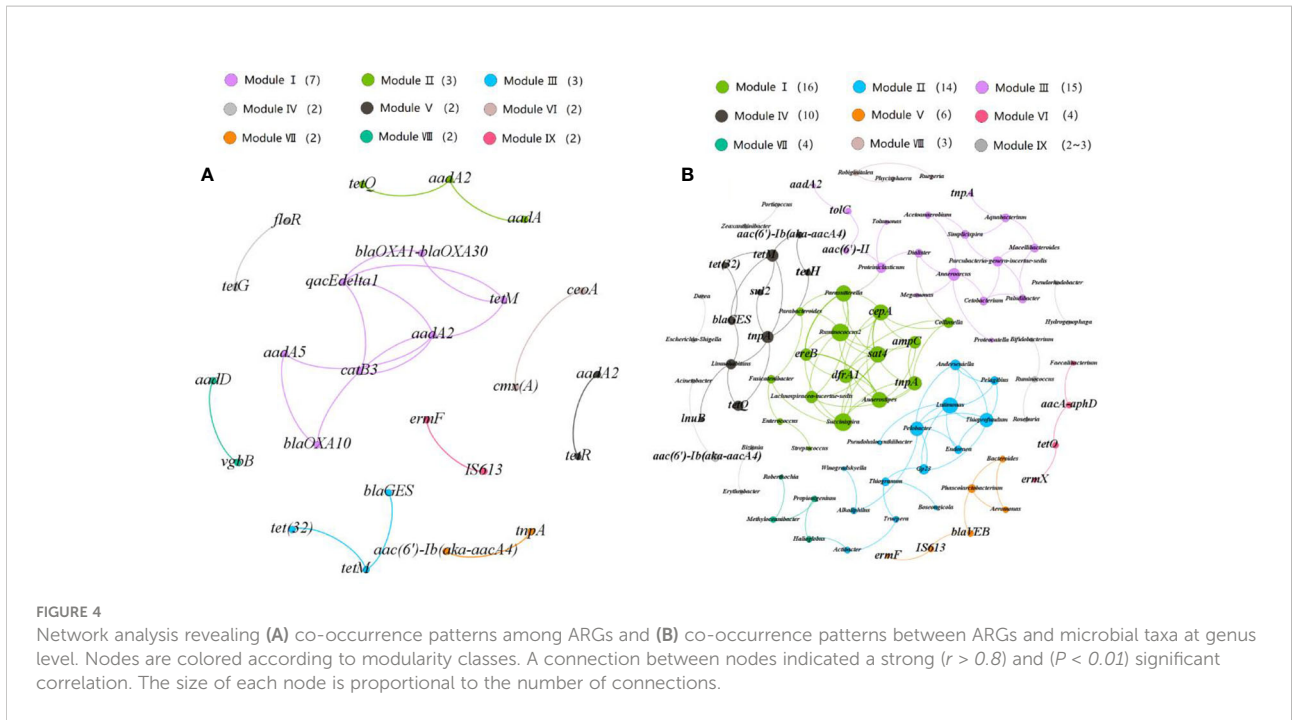
The relationship between microbial communities, MGEs, environmental factors, and ARGs were explored by RDA analysis (Figure 5A). Taken together, the selected environmental variables ( $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{PO}_4^{3-}$ , salinity, pH), MGEs, and bacterial communities explained 99.8% of the variation in ARGs. MGEs, salinity, and  $\text{NH}_4^+\text{-N}$  were significantly positively correlated with the first axis and ARGs from the estuaries of the Tang, Xinkai and Shi Rivers.  $\text{PO}_4^{3-}$ , pH,  $\text{NO}_3\text{-N}$ , *Litoreaibacter*, and *Psychrobacter* were significantly positively correlated with ARGs in the RCWs of the Xinkai, Nandai, and Yang Rivers. Partial RDA was performed to further distinguish the effects of environmental factors, MGEs and bacterial communities on antibiotic resistomes in water samples. Based on partial RDA, the bacterial communities accounted for 50.3% of the total ARG variations, much more than the sum of the contribution of MGEs (18.1%) and environmental factors (8.3%). The interaction between environmental factors and microbial community structure, as well as those between MGEs and the bacterial community accounted for 5.9% and 15.2% of total resistome variation, respectively. Mantel correlation based on Bray-Curtis distance showed significant correlations between bacterial communities and antibiotic resistomes in the coastal environments studied ( $r^2 = 0.4591$ ,  $p = 0.008$ ). The contributions of microbial communities, MGEs and environmental factors to the relative abundance of ARGs in the samples were further explored separately using ABT analysis. Figure S6 shows the relative

TABLE 1 Pearson's correlation analysis among the normalized abundances of ARGs and MGEs.

	MGEs	Integrans	Transposase
Aminoglycoside	0.78**	0.25	0.82**
Beta_Lactamase	0.15	0.54	0.027
Chloramphenicol	0.94**	0.32	0.98**
MLSB	0.99**	0.48	0.99**
Multidrug	0.73*	0.60	0.66*
Sulfonamide	0.67*	0.50	0.63
Tetracycline	0.98**	0.46	0.98**
Vancomycin	-0.04	0.33	-0.13

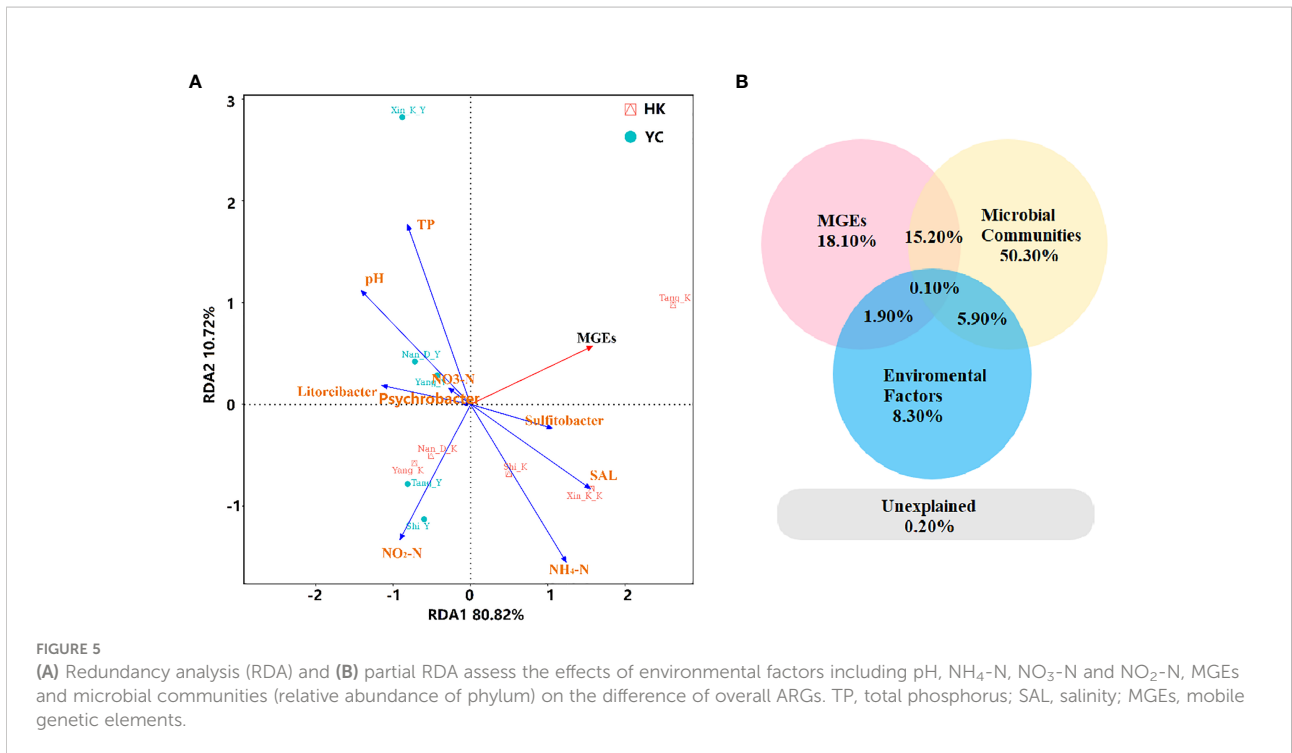
\* $p < 0.05$ , and \*\* $p < 0.01$ .





importance of these indicators. The greater the value, the higher the importance of an indicator. MGEs strongly influenced the occurrence of ARGs in the waters. In addition, the concentration of  $\text{NO}_2^-$  (0.011-0.02 mg/L) also had a strong effect on the relative abundance of ARGs. In terms of microbial communities, Acidobacteria had the greatest influence on the

resistomes in RCWs and their adjacent estuaries. Coexistence of multiples integrative and conjugative elements has been found in the chromosome of Acidobacteria (Gonçalves and Santana, 2021). Therefore, they could easily become resistant to antibiotics and contribute to the dissemination of ARGs, mediated by the MGEs in estuaries and RCWs.



## Discussion

### ARGs and MGEs were widely distributed in RCWs

An abundance of diverse ARGs are alarmingly widespread in estuarine environments. Our study detected a broad spectrum of ARGs in RCWs using HT-qPCR techniques, and their normalized copy numbers were close to, or even higher than, those in adjacent estuaries. In particular, 95 and 72 ARGs were detected in the RCWs adjacent to the estuaries of Tang and Xinkai rivers, respectively. The corresponding relative abundances were as high as 2.6 and 1.3/16S rRNA gene, respectively. Clearly, human exposure to these ARGs in RCWs (for example by aerosol inhalation, dermal, eye or ear contact, and oral intake) may represent potential health risks to those swimming in recreational bathing waters, despite there being no currently known infectious dose of antibiotic resistant bacteria or ARGs. In addition, waterborne pathogens also present major threats to public health. *Arcobacter* spp. were only identified in estuarine samples by the Illumina sequences of their 16S rRNA genes. Whether these species are pathogenic depends on their expression of a few virulence factors. There have been reports of the widespread occurrence of pathogenic marker genes in RCWs, such as the virulence factors of *Acanthamoeba* spp., *Clostridium perfringens*, enteropathogenic *Escherichia coli*, *Klebsiella pneumoniae*, *Vibrio cholerae/V. parahaemolyticus* and *Legionella* spp (An et al., 2020). Co-contamination of waters with pathogens and ARGs could probably contribute to the evolution antibiotic resistant pathogenic bacteria. Therefore, the routine monitoring of waterborne ARGs and pathogens is imperative to improve or safeguard human, animal and environmental health, as emphasized in the various “One-Health” initiatives.

The normalized copy numbers of ARGs detected in the RCWs and estuaries of the Tang and Xinkai rivers were even higher than those in some other environmental samples, such as aquaculture waters (P. Gao et al., 2012), estuarine sediments (Y. G. Zhu et al., 2017), and soils (Y. G. Zhu et al., 2013). The high abundance of ARGs in these two estuaries could be explained by the particularly dense populations in these watersheds. Intensive human activities, such as increased sewage discharge and large aquacultural developments might contribute to the relatively high abundance of ARGs in the Tang and Xinkai Rivers and their accumulation in the estuarine environments. Intensive human activity has been reported to facilitate the spread of ARGs, and population density has been positively correlated with the abundance of ARGs (Uyaguari et al., 2013; Berglund, 2015).

ARGs and MGEs associated with clinical setting have been commonly detected in coastal environments, and can often be traced back to particular hospitals and sewage discharges. For

example, vancomycin is only used as the “last line” in the treatment of human bacterial infections. Previous studies have detected the vancomycin resistant gene (*vanA*) in ancient permafrost sediments predating the use of antibiotics (D’Costa et al., 2011), indicating that it occurs naturally. In 2017, the normalized copy number of vancomycin in estuarine sediment around China was as high as  $9.3 \times 10^{-3}$ /16S rRNA genes (Zhu et al., 2017). We found vancomycin resistant genes in all of the estuaries and RCWs, with the relative abundances ranging from  $3.7 \times 10^{-4} \sim 2.4 \times 10^{-3}$  and  $1.5 \times 10^{-3} \sim 1.1 \times 10^{-1}$ /16S rRNA genes, respectively, which could be related to overuse of vancomycin or unregulated wastewater from hospitals or medical facilities. The growing occurrence and abundance of vancomycin resistance presents an urgent problem. The  $\beta$ -Lactamase *bla*TEM gene had the highest relative abundance in the samples, possibly due to overuse of the antibiotic cephalosporin in clinical settings. It is worth noticing that *bla*TEM gene has the potential to encode TEM-1  $\beta$ -lactamase, which requires only a few specific single nucleotide hydrolytic polymorphisms to evolve into a gene encoding an extended-spectrum  $\beta$ -lactamase that degrades all cephalosporins including ceftriaxone (Muhammad et al., 2014). Diversity of clinically relevant antimicrobial resistance genes was found in urban beaches. The clinical *int1* gene commonly detected in RCWs occurs in a variety of clinical pathogenic bacteria and in the human intestinal flora and can be used as a biomarker for municipal sewage pollution and is closely related to human health risks (Zheng et al., 2020). The results were in consistence with previously published paper indicating that diverse clinically relevant ARGs have been found in urban beaches (Furlan et al., 2021). Together, our study indicated that estuaries and RCWs may be polluted by medical and municipal sewage.

### ARGs patterns was strongly affected by bacterial phylogenetic compositions in coastal water environments

The RCW samples analyzed in this study were collected along the coastline of Qinhuangdao. However, the two estuaries with the highest normalized copy numbers (the Tang and Xinkai Rivers) were not adjacent to each other, and there was no overall spatial pattern in antibiotic resistomes in the estuarine areas. These results suggested that environmental factors such as tides or latitude did not the drive the occurrence of ARGs in these estuarine environments. Human activities is frequent in this area and people can either acquire ARGs through direct exposure when swimming there and also introduce new ARGs, so promoting their horizontal transfer of ARGs (Chaturvedi et al., 2021). In order to minimize the impact of human activities on the ARGs detected in RCWs, water samples were collected in winter to minimize the anthropogenic variation in the data. ARGs profiles (qPCR data) were significantly correlated

with bacterial community (16S rRNA genes, OTU data), as shown by the Mantel test. Environmental factors (NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, NO<sub>2</sub><sup>-</sup>-N, PO<sub>4</sub><sup>3-</sup>, salinity, pH), bacterial community compositions (relative abundance of major bacterial phyla), and MGEs accounted for 99.8% of total variation in ARGs profiles in the RCWs. Separation of the variations explained by subsets of these factors indicated that MGEs accounted for 18.1% of the subset variation, while bacterial compositions accounted for a much higher percentage of the variation in ARGs (50.3%, Figure 5B). These data indicated that the particular microbial community was a driving force in the variation of ARGs in these estuarine areas. Thus, any factors that disturb the microbial community, such as salinity, nutrient level, organic pollutants, and storm and runoff levels, may exert previously underestimated increases in abundances of ARGs.

MGEs are vehicles for horizontal transfer of ARGs, and have been shown to promote transmission and enrichment of ARGs in many other environments (Wright et al., 2008; Hoa et al., 2011; Zhang et al., 2021). In this study, MGEs accounted for 18.1% of the total variation in ARGs according to the pRDA analysis. Based on the network analysis of ARGs, MGEs, and microbial genera, the correlation between transposons (TnpA) and aminoglycoside resistant gene (aac(6′)-Ib(aka aacA4)) indicated that conjugation may play a role in the dissemination of ARGs in RCWs. Transposons can transfer themselves and related resistant genes at random to a new location within the cell, including plasmids, while integrons can transfer resistant genes between defined sites by site-specific recombination (Cox and Wright, 2013). Although no correlations between ARGs and integrons were detected, the role of MGEs in the enrichment of ARGs in estuarine areas should not be overlooked. It is noteworthy that *Acinetobacter*, an opportunistic pathogen, was also significantly associated with the aac(6′)-Ib(aka aacA4) gene and may express aminoglycoside resistance. *Acinetobacter* may cause lung infection through oral inhalation or direct exposure (Wong et al., 2017). In recent years, the occurrence of antibiotic resistant *Acinetobacter* has been increasing, and ampicillin resistance has remained at a relatively high level (Kutlu et al., 2008). Previous studies have also reported marker genes for *Acinetobacter baumannii* AB0057 and *Acinetobacter oleivorans* DR1 in marine recreational beaches around Xiamen City, China (An et al., 2020). Our study showed that coastal environments are ideal places for bacteria or even pathogens to acquire ARGs, and that recreational activities in coastal water may put human beings at risk of antibiotic resistant bacteria infections.

## Impacts of estuaries on adjacent recreational coastal waters

PCoA and hierarchical cluster analysis based on Bray-Curtis distance revealed the consistency of ARG occurrences between

estuaries and their corresponding RCWs, indicating that estuaries may, to some extent, impact the ARGs in adjacent bathing waters through currents or water exchange. The “Qinhuangdao Recreational Coastal Water Management Regulations”, established to avoid contamination from estuaries, state that the RCWs should be located at least 500 m away from an estuary. However, this distance cannot totally avoid the potential threat of ARGs. ARGs from livestock farms, municipal sewage, medical wastewater, and agricultural sewage outlets may spread to bathing waters through estuaries and threaten human health. Previous studies have reported that if RCWs located downstream from poultry farms or municipal sewage discharge points, people swimming are likely to be exposed to *Escherichia coli* expressing broad-spectrum β-lactamase (Schijyen et al., 2015). Besides, the diverse and abundant ARGs in RCWs could be due to the fact that frequent human activities contribute to permanent changes in properties of RCWs. For example, changes in environmental factors such as differences in organic matter level (Lin et al., 2019), total nitrogen (J. Chen et al., 2019), and temperature (Liao et al., 2018) have been proven to drive the shift of bacterial communities and promote the propagation of ARGs in aquatic environments. Based on ABT analysis, the concentration of NO<sub>2</sub><sup>-</sup> also had an important effect on the relative abundance of ARGs. We found that NO<sub>2</sub><sup>-</sup>-N in RCWs was generally higher than in estuaries. According to Cen et al. (2020), nitrite higher than 1 mg/ml could stimulate free radical-induced RpoS regulators and SOS responses, increase cell membrane permeability, and regulate binding transfer-related genes, thus promoting the horizontal transfer of ARGs. Together, these results indicated that multiple factors could result in the propagation of ARGs and further emphasize the importance of routine surveillance of ARGs in RCWs.

## Conclusions

The results of this study showed that the abundance of ARGs in RCWs ranged from 9.9×10<sup>-2</sup> to 2.1/16S rRNA gene, higher than in many other environments such as soil, estuarine sediments, and aquacultural developments. The consistency between RCW and their adjacent estuaries indicated that estuaries, to some extent, may have an impact on ARGs in RCWs. Thus, the current rules regulating RCWs cannot avoid the threat of ARGs to human health, and humans may be exposed to ARGs when swimming in RCWs. The Mantel test suggested that microbial community structure is the main force driving the occurrence of ARGs in coastal water environment. Besides the selective pressure of antibiotics, attention should be paid to the spread and enrichment of ARGs that may be overlooked when any perturbation disturbs the microbial composition of swimming waters, such as changes in

temperatures, N and P levels, or even algal abundances. Moreover, the consistency between antibiotic resistomes in RCWs and their adjacent estuaries suggested that ARGs from estuaries could contaminate adjacent RCWs. Therefore, proper regulations of bathing areas and reducing the discharge of ARG into terrigenous runoff may be useful in controlling ARG levels in RCWs, and so control the risks to public health. More work is needed to determine the infectious concentrations and risk levels of ARGs to better assess the potential public health risk of ARGs in RCWs. ARG levels and microbial community composition in bathing waters should be routinely detected in real-time.

## Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#). Further inquiries can be directed to the corresponding author.

## Author contributions

YH: Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. YKH: Formal analysis, Conceptualization, Writing – review & editing. HXW: Conceptualization, Formal analysis, Writing – review & editing. YWL: Writing – review & editing. JYW: Data curation. YCZ: Methodology. HW: Writing – review & editing. All authors contributed to the article and approved the submitted version.

## References

- Ali, L., Wang, Y. Q., Zhang, J., Ajmal, M., Xiao, Z. R., Wu, J. R., et al. (2016). Nutrient-induced antibiotic resistance in enterococcus faecalis in the eutrophic environment. *J. Glob. Antimicrob. Resist.* 7, 78–83. doi: 10.1016/j.jgar.2016.07.014
- An, X. L., Wang, J. Y., Pu, Q., Li, H., Pan, T., Li, H. Q., et al. (2020). High-throughput diagnosis of human pathogens and fecal contamination in marine recreational water. *Environ. Res.* 190, 109982. doi: 10.1016/j.envres.2020.109982
- Berglund, B. (2015). Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics. *Infect. Ecol. Epidemiol.* 5, 28564–28564. doi: 10.3402/iee.v5.28564
- Bigus, K., Astel, A., and Niedzielski, P. (2016). Seasonal distribution of metals in vertical and horizontal profiles of sheltered and exposed beaches on polish coast. *Mar. Pollut. Bull.* 106 (1–2), 347–359. doi: 10.1016/j.marpolbul.2016.03.009
- Bi Weihong, Li Yu, Jiancheng, S., Yajie, J., Pingjie, H., and Fei, Xu (2022). Research review and development trend of marine total organic carbon detection based on spectral technology. *J. YanShan Univ.* 46 (1), 1–8. doi: 10.3969/j.issn.1007-791X.2022.01.001
- Blahova, J., Kralikova, K., and Krcmery, V. (1999). Reservoirs, interactions and stability of genetic resistance to antibiotics. the "easy to get–hard to lose" syndrome. [Rezervory, interakcie a stabilita genov rezistence na antibiotika. syndrom "easy to get–hard to lose"]. *Cas. lek. Cesk.* 138 (14), 424–428.
- Cen, T., Zhang, X., Xie, S., and Li, D. (2020). Preservatives accelerate the horizontal transfer of plasmid-mediated antimicrobial resistance genes via differential mechanisms. *Environ. Int.* 138, 105544. doi: 10.1016/j.envint.2020.105544
- Chaturvedi, P., Chowdhary, P., Singh, A., Chaurasia, D., Pandey, A., Chandra, R., et al. (2021). Dissemination of antibiotic resistance genes, mobile genetic elements, and efflux genes in anthropogenically impacted riverine environments. *Chemosphere* 273, 129693. doi: 10.1016/j.chemosphere.2021.129693
- Chen, J., Deng, W. J., Liu, Y. S., Hu, L. X., He, L. Y., Zhao, J. L., et al. (2019). Fate and removal of antibiotics and antibiotic resistance genes in hybrid constructed wetlands. *Environ. Pollut.* 249, 894–903. doi: 10.1016/j.envpol.2019.03.111
- Chen, H., and Zhang, M. M. (2013). Effects of advanced treatment systems on the removal of antibiotic resistance genes in wastewater treatment plants from hangzhou, China. *Environ. Sci. Technol.* 47 (15), 8157–8163. doi: 10.1021/es401091y
- Cox, G., and Wright, G. D. (2013). Intrinsic antibiotic resistance: Mechanisms, origins, challenges and solutions. *Int. J. med. Microbiol. Int. J. Med. Microbiol.* 303 (6–7), 287–292. doi: 10.1016/j.ijmm.2013.02.009
- Culliton, T. J. (1998) *Population: Distribution, density, and growth*. Available at: [https://www.researchgate.net/publication/247207344\\_Population\\_Distribution\\_Density\\_and\\_Growth](https://www.researchgate.net/publication/247207344_Population_Distribution_Density_and_Growth).

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary Material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2022.976438/full#supplementary-material>



- D'Costa, V. M., King, C. E., Kalan, L., Morar, M., Sung, W. W. L., Schwarz, C., et al. (2011). Antibiotic resistance is ancient. *Nature* 377 (7365), 457–461. doi: 10.1038/nature10388
- Dalcin, E., and Jackson, P. W. (2018). A network-wide visualization of the implementation of the global strategy for plant conservation in Brazil. *Rodriguésia*. 69 (4), 1613–1639. doi: 10.1590/2175-7860201869411
- Forsberg, K. J., Patel, S., Gibson, M. K., Lauber, C. L., Knight, R., Fierer, N., et al. (2014). Bacterial phylogeny structures soil resistomes across habitats. *Nature*. 509 (7502), 612–615. doi: 10.1038/nature13377
- Freeman, C. N., Scriver, L., Neudorf, K. D., Hansen, L. T., and Jamieson, R. C. (2018). Antimicrobial resistance gene surveillance in the receiving waters of an upgraded wastewater treatment plant. *Facets* 3, 128–138. doi: 10.1139/facets-2017-0085
- Furlan, J. P. R., Ramos, M. S., dos Santos, L. D. R., Gallo, I. F. L., Lopes, R., and Stehling, E. G. (2021). Appearance of *mcr-9*, *blaKPC*, *cfp* and other clinically relevant antimicrobial resistance genes in recreation waters and sands from urban beaches, Brazil. *Mar. pollut. Bull.* 167, 112334. doi: 10.1016/j.marpolbul.2021.112334
- Gao, F. L., Li, J. X., Hu, J., Sui, B. L., Wang, C. A. X., Sun, C. J., et al. (2021). The seasonal distribution characteristics of microplastics on bathing beaches along the coast of qingdao, China. *Sci. Total Environ.* 783, 146969. doi: 10.1016/j.scitotenv.2021.146969
- Gao, P., Mao, D., Luo, Y., Wang, L., Xu, B., and Xu, L. (2012). Occurrence of sulfonamide and tetracycline-resistant bacteria and resistance genes in aquaculture environment. *Water Res.* 46 (7), 2355–2364. doi: 10.1016/j.watres.2012.02.004
- Gonçalves, O. S., and Santana, M. F. (2021). The coexistence of monopartite integrative and conjugative elements in the genomes of acidobacteria. *Gene* 777, 145476. doi: 10.1016/j.gene.2021.145476
- Hoa, P. T. P., Managaki, S., Nakada, N., Takada, H., Shimizu, A., Anh, D. H., et al. (2011). Antibiotic contamination and occurrence of antibiotic-resistant bacteria in aquatic environments of northern Vietnam. *Sci. Total Environ.* 409 (15), 2894–2901. doi: 10.1016/j.scitotenv.2011.04.030
- Huang, H. W., Zeng, S. Y., Dong, X., Li, D., Zhang, Y., He, M., et al. (2019). Diverse and abundant antibiotics and antibiotic resistance genes in an urban water system. *J. Environ. Manage.* 231, 494–503. doi: 10.1016/j.jenvman.2018.10.051
- Huang, H. N., Zheng, X., Chen, Y. G., Liu, H., Wan, R., and Su, Y. L. (2017). Alkaline fermentation of waste sludge causes a significant reduction of antibiotic resistance genes in anaerobic reactors. *Sci. Total Environ.* 580, 380–387. doi: 10.1016/j.scitotenv.2016.11.186
- Jacoby, G. A. (2006).  $\beta$ -lactamase nomenclature nomenclature. *Antimicrob. Agents Chemother.* 50 (4), 1123–1129. doi: 10.1128/AAC.50.4.1123-1129.2006
- Jang, H. M., Kim, Y. B., Choi, S., Lee, Y., Shin, S. G., Unno, T., et al. (2018). Prevalence of antibiotic resistance genes from effluent of coastal aquaculture, south Korea. *Environ. pollut.* 233, 1049–1057. doi: 10.1016/j.envpol.2017.10.006
- Kutlu, S. S., Sacar, S., Suzer, T., Cevahir, N., Okke, D., Caylak, S. D., et al. (2008). Successful treatment of a patient with multidrug resistant acinetobacter baumannii meningitis with high dose ampicillin-sulbactam. *Mikrobiyol. Bul.* 42 (2), 353–358.
- Liao, H., Lu, X., Rensing, C., Friman, V. P., Geisen, S., Chen, Z., et al. (2018). Hyperthermophilic composting accelerates the removal of antibiotic resistance genes and mobile genetic elements in sewage sludge. *Environ. Sci. Technol.* 52 (1), 266–276. doi: 10.1021/acs.est.7b04483
- Lin, W. F., Wan, K., Zeng, J., Li, J. J., Li, X., and Yu, X. (2019). Low nutrient levels as drinking water conditions can reduce the fitness cost of efflux pump-mediated ciprofloxacin resistance in *Pseudomonas aeruginosa*. *J. Environ. Sci.(China)*. 83, 123–132. doi: 10.1016/j.jes.2019.03.022
- Magoc, T., and Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*. 27 (21), 2957–2963. doi: 10.1093/bioinformatics/btr507
- Marti, E., Variatza, E., and Balcazar, J. L. (2014). The role of aquatic ecosystems as reservoirs of antibiotic resistance. *Trends Microbiol.* 22 (1), 36–41. doi: 10.1016/j.tim.2013.11.001
- Muhammad, I., Golparian, D., Dillon, J. A. R., Johansson, A., Ohnishi, M., Sethi, S., et al. (2014). Characterisation of bla(TEM) genes and types of beta-lactamase plasmids in neisseria gonorrhoeae - the prevalent and conserved bla(TEM-135) has not recently evolved and existed in the Toronto plasmid from the origin. *BMC Infect. Dis. BMC Infect. Dis.* 14, 454. doi: 10.1186/1471-2334-14-454
- O'Neil, J. (2014) Review on antimicrobial resistance antimicrobial resistance: tackling a crisis for the health and wealth of nations. In: *Review on antimicrobial resistance*. Available at: [https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations\\_1.pdf](https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf) (Accessed 9 March 2022).
- Ouyang, W. Y., Huang, F. Y., Zhao, Y., Li, H., and Su, J. Q. (2015). Increased levels of antibiotic resistance in urban stream of jiulongjiang river, China. *Appl. Microbiol. Biotechnol.* 99 (13), 5697–5707. doi: 10.1007/s00253-015-6416-5
- Pruden, A., Pei, R. T., Storteboom, H., and Carlson, K. H. (2006). Antibiotic resistance genes as emerging contaminants: Studies in northern Colorado. *Environ. Sci. Technol.* 40 (23), 7445–7450. doi: 10.1021/es060413l
- Qinhuangdao Municipal People's Government (2019) *Regulations of qinhuangdao municipality on the administration of bathing beaches*. Available at: [http://www.qhd.gov.cn/front\\_pcthi.do?uuiid=8F0D6662565323FFB03AC4C9AE146E42](http://www.qhd.gov.cn/front_pcthi.do?uuiid=8F0D6662565323FFB03AC4C9AE146E42) (Accessed 9 March 2022).
- Rider, B. F., and Mellon, M. G. (1946). Colorimetric determination of nitrites. *Ind. Eng. Chem. Analytical Ed.* 18 (2), 96–99. doi: 10.1021/i560150a003
- Rodriguez-Mozaz, S., Chamorro, S., Marti, E., Huerta, B., Gros, M., Sanchez-Melsio, A., et al. (2015). Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. *Water Res.* 69, 234–242. doi: 10.1016/j.watres.2014.11.021
- Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahe, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4, e2584. doi: 10.7717/peerj.2584
- Sarkar, S. K., and Chang, C. K. (1997). The simes method for multiple hypothesis testing with positively dependent test statistics. *J. Am. Stat. Assoc.* 92 (440), 1601–1608. doi: 10.2307/2965431
- Schiyyen, J. F., Blaak, H., Schets, F. M., Husman, A. M., and d, R. (2015). Fate of extended-spectrum beta-Lactamase-Producing escherichia coli from faecal sources in surface water and probability of human exposure through swimming. *Environ. Sci. Technol.* 49 (19), 11825–11833. doi: 10.1021/acs.est.5b01888
- Stepanuskas, R., Glenn, T. C., Jagoe, C. H., Tuckfield, R. C., Lindell, A. H., King, C. J., et al. (2006). Coselection for microbial resistance to metals and antibiotics in freshwater microcosms. *Environ. Microbiol.* 8 (9), 1510–1514. doi: 10.1111/j.1462-2920.2006.01091.x
- Stewart, J. R., Gast, R. J., Fujioka, R. S., Solo-Gabriele, H. M., Meschke, J. S., Amaral-Zettler, L. A., et al. (2008). The coastal environment and human health: microbial indicators, pathogens, sentinels and reservoirs. *Environ. Health* 7, s3. doi: 10.1186/1476-069x-7-s2-s3
- Su, J., Fan, J., Ming, H., Guo, G., Fu, Y., Zhao, X., et al. (2022). The municipal sewage discharge may impact the dissemination of antibiotic-resistant escherichia coli in an urban coastal beach. *Water* 14, 1639. doi: 10.3390/w14101639
- Sun, J. T., Jin, L., He, T. T., Wei, Z., Liu, X. Y., Zhu, L. Z., et al. (2020). Antibiotic resistance genes (ARGs) in agricultural soils from the Yangtze river delta, China. *Sci. Total Environ.* 740, 140001. doi: 10.1016/j.scitotenv.2020.140001
- Su, J. Q., Wei, B., Ouyang, W. Y., Huang, F. Y., Zhao, Y., Xu, H. J., et al. (2015). Antibiotic resistome and its association with bacterial communities during sewage sludge composting. *Environ. Sci. Technol.* 49 (12), 7356–7363. doi: 10.1021/acs.est.5b01012
- Tamminen, M., Karkman, A., Lohmus, A., Muziasari, W. I., Takasu, H., Wada, S., et al. (2011). Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure. *Environ. Sci. Technol.* 45 (2), 386–391. doi: 10.1021/es102725n
- Tandukar, M., Oh, S., Tezel, U., Konstantinidis, K. T., and Pavlostathis, S. G. (2013). Long-term exposure to benzalkonium chloride disinfectants results in change of microbial community structure and increased antimicrobial resistance. *Environ. Sci. Technol.* 47 (17), 9730–9738. doi: 10.1021/es401507k
- Tomova, A., Ivanova, L., Buschmann, A. H., Rioseco, M. L., Kalsi, R. K., Godfrey, H. P., et al. (2015). Antimicrobial resistance genes in marine bacteria and human uropathogenic escherichia coli from a region of intensive aquaculture. *Environ. Microbiol. Rep.* 7 (5), 803–809. doi: 10.1111/1758-2229.12327
- Uyaguari, M. I., Scott, G. I., and Norman, R. S. (2013). Abundance of class 1-3 integrons in south Carolina estuarine ecosystems under high and low levels of anthropogenic influence. *Mar. pollut. Bull.* 76 (1-2), 77–84. doi: 10.1016/j.marpolbul.2013.09.027
- von Wintersdorff, C. J. H., Penders, J., van Niekerk, J. M., Mills, N. D., Majumder, S., van Alphen, L. B., et al. (2016). Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Front. Microbiol.* 7. doi: 10.3389/fmicb.2016.00173
- Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73 (16), 5261–5267. doi: 10.1128/aem.00062-07
- Wang, Y. Q., Lu, S. Y., Liu, X. H., Chen, J., Han, M. Z., Wang, Z., et al. (2021). Profiles of antibiotic resistance genes in an inland salt-lake ebinur lake, xinjiang, China: The relationship with antibiotics, environmental factors, and microbial communities. *Ecotoxicol. Environ. Saf.* 221, 112427. doi: 10.1016/j.ecoenv.2021.112427
- Wei, Z. L., Miao, J., Yang, Z. W., Shi, D. Y., Wu, H. Y., Yang, D., et al. (2020). Contamination sources of the enteric virus in recreational marine water shift in a seasonal pattern. *Sci. Total Environ.* 743, 140641. doi: 10.1016/j.scitotenv.2020.140641
- Wen, H.-q., Shi, J., Xun, H., and Deng, H. P. (2015). Distribution, dissemination and removal of antibiotic resistant genes (ARGs) in the aquatic environment. *J. Appl. Ecol.* 26 (2), 625–635.



- Wong, D., Nielsen, T. B., Bonomo, R. A., Pantapalangkoor, P., Luna, B., and Spellberg, B. (2017). Clinical and pathophysiological overview of acinetobacter infections: a century of challenges. *Clin. Microbiol. Rev.* 30 (1), 409–447. doi: 10.1128/cmr.00058-16
- Wright, M. S., Baker-Austin, C., Lindell, A. H., Stepanauskas, R., Stokes, H. W., and McArthur, J. V. (2008). Influence of industrial contamination on mobile genetic elements: class 1 integron abundance and gene cassette structure in aquatic bacterial communities. *ISME J.* 2 (4), 417–428. doi: 10.1038/ismej.2008.8
- Wu, D., Ma, R. Q., Wei, H. W., Yang, K., and Xie, B. (2018). Simulated discharge of treated landfill leachates reveals a fueled development of antibiotic resistance in receiving tidal river. *Environ. Int.* 114, 143–151. doi: 10.1016/j.envint.2018.02.049
- Wu, B., Song, J. M., and Li, X. G. (2014). Evaluation of potential relationships between benthic community structure and toxic metals in laizhou bay. *Mar. Pollut. Bull.* 87 (1-2), 247–256. doi: 10.1016/j.marpolbul.2014.07.052
- Yang, J., Wang, H., Roberts, D. J., Du, H. N., Yu, X. F., Zhu, N. Z., et al. (2020). Persistence of antibiotic resistance genes from river water to tap water in the Yangtze river delta. *Sci. Total Environ.* 742, 140592. doi: 10.1016/j.scitotenv.2020.140592
- Yang, J., Wang, C., Shu, C., Liu, L., Geng, J., Hu, S., et al. (2013). Marine sediment bacteria harbor antibiotic resistance genes highly similar to those found in human pathogens. *Microb. Ecol.* 65 (4), 975–981. doi: 10.1007/s00248-013-0187-2
- Zhang, Y. J., Hu, H. W., Yan, H., Wang, J. T., Lam, S. K., Chen, Q. L., et al. (2019). Salinity as a predominant factor modulating the distribution patterns of antibiotic resistance genes in ocean and river beach soils. *Sci. Total Environ.* 668, 193–203. doi: 10.1016/j.scitotenv.2019.02.454
- Zhang, T., Li, J., Wang, N., Wang, H., and Yu, L. (2021). Metagenomic analysis reveals microbiome and resistome in the seawater and sediments of kongsfjorden (Svalbard, high Arctic). *Sci. Total Environ.* 809, 151937. doi: 10.1016/j.scitotenv.2021.151937
- Zhang, K., Xin, R., Zhao, Z., Li, W. P., Wang, Y. N., Wang, Q., et al. (2021). Mobile genetic elements are the major driver of high antibiotic resistance genes abundance in the upper reaches of huaihe river basin. *J. Hazard. Mater.* 401, 123271. doi: 10.1016/j.jhazmat.2020.123271
- Zheng, W. L., Huyan, J. Q., Tian, Z., Zhang, Y., and Wen, X. H. (2020). Clinical class 1 integron-integrase gene - a promising indicator to monitor the abundance and elimination of antibiotic resistance genes in an urban wastewater treatment plant. *Environ. Int.* 135, 105372. doi: 10.1016/j.envint.2019.105372
- Zhuang, M., Achmon, Y., Cao, Y. P., Liang, X. M., Chen, L., Wang, H., et al. (2021). Distribution of antibiotic resistance genes in the environment star. *Environ. Pollut.* 285, 117402. doi: 10.1016/j.envpol.2021.117402
- Zhu, Y. G., Johnson, T. A., Su, J. Q., Qiao, M., Guo, G. X., Stedtfeld, R. D., et al. (2013). Diverse and abundant antibiotic resistance genes in Chinese swine farms. *P. Natl. Acad. Sci. U.S.A.* 110 (9), 3435–3440. doi: 10.1073/pnas.1222743110
- Zhu, L. H., Wu, J. Z., Xu, Z. Q., Xu, Y. C., Lin, J., and Hu, R. J. (2014). Coastline movement and change along the bohai Sea from 1987 to 2012. *J. Appl. Remote. Sens.* 8, 083585. doi: 10.1117/1.jrs.8.083585
- Zhu, Y. G., Zhao, Y., Li, B., Huang, C. L., Zhang, S. Y., Yu, S., et al. (2017). Continental-scale pollution of estuaries with antibiotic resistance genes. *Nat. Microbiol.* 2 (4), 7. doi: 10.1038/nmicrobiol.2016.270