

Characteristics of antibiotic resistance genes and microbial communities in marine sediments in seas of China

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Research Article

Keywords: Antibiotic resistance gene, Antibiotic resistance bacteria, Marine sediment, Environmental factors

Posted Date: May 18th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1661333/v1>

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Abstract

Marine sediments have been regarded as hotspots of antibiotic resistance genes (ARGs) due to the continuous release of antibiotics and ARGs into seas. A comprehensive study on the spatial distribution of ARGs in sediments from different seas is needed for a better understanding of ARGs pollution in China. Marine sediments were collected from the Bohai Sea, the Yellow Sea, the East China Sea, and the South China Sea, and five prevalent ARGs (i.e., *bla*_{TEM}, *sul1*, *tetA*, *tetC*, and *vanA*) and one class 1 integron-integrase gene (*int1*) were targeted and determined. β -lactam resistance genes *bla*_{TEM} were ubiquitous and predominant in all sea areas with higher relative abundances up to 2.7×10^{-3} , followed by sulfonamide (*sul1*) and tetracycline resistance genes (*tetC*). *Int1* genes were detected in all sediments except for one sampling site in the South China Sea, with relative abundances ranging from 3.6×10^{-5} to 1.3×10^{-3} . Total ARGs were more abundant in the South China Sea, followed by the Yellow Sea, the East China Sea, and the Bohai Sea. Microbial community analysis showed that the microbial community in sediments collected from different seas were clustered separately and appeared distinct spatial characteristics. Contents of nitrate, organic carbon, and total carbon are the major physicochemical properties that influence the distribution and variation of *bla*_{TEM} and *tetC* in sediments with their *p*-value lower than 0.05. Co-occurrence network analysis revealed that Bacteroidetes and Firmicutes were dominant potential host bacteria of *bla*_{TEM}, *tetC*, *sul1*, and *int1*. This study provides new insight into the pollution status of ARGs in the seas of China, which contributes to the ARGs risk assessment and raises awareness of managing antibiotic resistance pollution.

1. Introduction

Antibiotic resistance genes (ARGs) have been recognized as emerging environmental contaminants, posing a crucial threat to public health (Hendriksen et al., 2019). ARGs could be widespread transferred and disseminated via horizontal gene transfer (von Wintersdorff et al., 2016; Zhang et al., 2021), leading to the development of super antibiotic resistance bacteria that negates antibiotics. China is the largest manufacturer and consumer of antibiotics in the world with an amount of 150,000–20,000 t/yr, and up to 70% of the antibiotics used were released into the environment (Wu et al., 2015; Zhang et al., 2015). The intensive use of antibiotics in hospitals and animal husbandry is the major driving force for the occurrence of ARGs in natural environments (Zhang et al., 2019). ARGs were frequently detected in soil, sediments, water, and even air (Allen et al., 2010; Gaviria-Figueroa et al., 2019; Hendriksen et al., 2019; Urra et al., 2019). High abundances of resistance genes against tetracycline and sulfonamide (10^3 - 10^6 copies/mL) have been detected in medical treatment systems, wastewater treatment systems, and aquaculture wastewater (Gao et al., 2018; Hendriksen et al., 2019). The prevalence and proliferation of ARGs in the aquatic environment resulted in the entrance of ARGs into the marine environment (Lu et al., 2019a).

Marine not only play important roles in nitrogen and carbon cycling, and they were also regarded as reservoirs of aquatic and terrigenous pollutants (Chen et al., 2019a). Land-based pollution, including

wastewater discharge from industry, agriculture, fishery, and tourism, contributed to about 80% of the total pollution in the sea (Lu et al., 2019a). Marine has suffered from biodiversity changes during the last two centuries as a result of human activities including nutrient and contaminant discharge (Han et al., 2020; Worm et al., 2006). Occurrences of ARGs have been reported in the seas (Chen et al., 2019b; Lu et al., 2019a; Zhang et al., 2020c) and estuaries (Guo et al., 2018; Zhang et al., 2018b) in China. Various ARGs were targeted in different studies with diverse primers, which made comprehensive evaluation between different areas infeasible and inauthentic. In addition, ARGs are regarded as a complex problem with interconnected and multiple drivers, which might include climate change, temporal variation, trade, and populations (Hendriksen et al., 2019). The occurrence and spread of ARGs were reported to be enhanced under exposure to the metal (Lu et al., 2020), residue chlorine (Zhang et al., 2021), sweeteners (Yu et al., 2021), and non-antibiotic pharmaceuticals (e.g., ibuprofen and propranolol) (Wang et al., 2020b; Wang et al., 2021). Given the potential threat of ARGs to the health of human beings and marine creatures, it is of great importance to systematically evaluate ARGs in the seas of China. The effects of environmental and geographical factors on the occurrence and distribution of ARGs in marine sediments are worth exploring.

The coastal area of China is the most populated area in China, where most of the large cities are located. Intensive population and large-scale development of aquaculture in the coastal area in China have put marine water under the stress of antibiotics pollution, which subsequently incurs the development of ARGs. This study aims to explore the spatial distribution of ARGs in seas along the coastline of China and assess the correlation between the environmental factors and the distribution of ARGs. To achieve this objective, marine sediments from the Bohai Sea, the Yellow Sea, the East China Sea, and the South China Sea were collected to evaluate the occurrence of ARGs. Five prevalent ARGs (i.e., β -lactam resistance gene *bla*_{TEM}, sulfonamide resistance gene *su1*, tetracycline resistance genes *tetA* and *tetC*, and vancomycin resistance gene *vanA*) and one class 1 integron-integrase gene (*int1*) recognized as an indicator of horizontal gene transfer in the marine sediments were quantified and the microbial community in the sediments was characterized. Correlation analysis and Mantel test were performed to unravel the correlation between the occurrence of ARGs and environmental factors. Furthermore, potential host bacteria of ARGs were revealed based on co-occurrence network analysis. These findings would shed light on the assessment of antimicrobial resistance pollution in sea areas of China.

2. Materials And Methods

2.1 Study area and sample collection

In the present study, a total of twelve marine sediments were collected from four seas of China: NS-25, NS-26, NS-27 from the Bohai Sea (BS); YS-7, YS-9, YS-11 from the Yellow Sea (YS); S01-1, S01-3, S01-5 from the East China Sea (ECS); and Q02, Q10, DZ1 from the South China Sea (SCS) (Fig. 1). Surface sediment samples were collected using a box corer during the voyage by the research vessel "XIANGYANG 18". The samples of BS and YS were collected in November 2020, and the sampling from

ECS and SCS was conducted in May 2020 and August 2020 respectively. At each site, 3 ~ 5 replicate sediment cores were collected randomly and immediately sealed within sterile plastic bags, and stored at 4°C. On arrival at the lab, sediment cores of each site were thoroughly homogenized to form the final composite sediment sample under anaerobic conditions, and then each was divided into two parts: one part was used to determine physicochemical characteristics of the sediments, while the other part was stored at -20°C for molecular analyses.

2.2 Analyses of environmental variables

The environmental variables of the sediments collected from different sampling sites were characterized. The inorganic nitrogen contents, including ammonium and nitrate, were determined spectrophotometrically according to the standard method (APHA, 2005). The total nitrogen (TN) was analyzed using the digestion method with hydrogen peroxide and sulfuric acid as described previously (Liu et al., 2015a). The pH and the organic content of the sediments were determined using previously described methods (Baolan et al., 2014).

2.3 Extraction of DNA and quantification of ARGs

The total genomic DNA of samples was extracted using the TIANNAMP Soil DNA Kit (TIANGEN, China) according to the manufacturer's instructions. The abundance of five prevalent ARGs including *bla*_{TEM}, *sul1*, *tetA*, *tetC*, and *vanA*, and a class 1 integron-integrase gene (*intI1*) were determined. Quantification of target ARGs, *intI1*, and 16S rRNA genes in the extracted DNA were performed by a StepOnePlus™ Real-Time PCR system (Thermo, USA). Briefly, qPCR was carried out in a system with a total volume of 10 μL, consisting of 5 μL of 2 × TB Green Premix Ex Taq II (Tli RNaseH Plus), 0.4 μL forward primer, 0.4 μL reverse primer, 0.2 μL of 50 × ROX Reference Dye (50 ×), 1.0 μL DNA sample, and 3.0 μL ddH₂O. The PCR reaction was conducted following: 95°C for 30 s, 40 cycles of 95°C for 5 s, and 40 cycles of 60°C for 30 s. The detection limit was set to be at a threshold cycle (CT) of 40. The relative abundances of ARGs and *intI1* were determined to eliminate the influence of biomass and calculated as the copy ratio of the target genes and the 16S rRNA genes (Wu et al., 2015; Zhang et al., 2021).

2.4 Microbial community revealed by 16S rRNA gene amplicon sequencing

The microbial communities of samples were analyzed via 16S rRNA gene amplicon sequencing. The 16S rRNA genes of bacteria were amplified using the universal primer set of 314F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGG TATCTAAT-3'). The PCR reaction was conducted with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers, and 10 ng template DNA. The PCR reaction was performed with the following program: 1 min at 98°C, 30 cycles at 98°C for 10 s, 30 s at 60°C, 30 s at 72°C, and finally 72°C for 5 min. Afterward, the PCR products were mixed with the same amount of loading buffer (containing SYB green), and the electrophoresis detection was operated on agarose gel (2%). The mixture was then purified by Qiagen Gel Extraction Kit (Qiagen, Germany) and sequenced at Novegene, Inc (Beijing, China). Quality filtering on the raw tags was

conducted under specific filtering conditions to obtain high-quality and clean tags using QIIME (V1.9.1). The taxonomy of OTUs was assigned against SILVA (version 132) database.

2.5 Statistical analysis

Mantel tests based on Euclidean distance were conducted to identify the environmental factors that were significantly correlated to the community composition (based on the relative abundance of all OTUs) (Mantel, 1985). The potential hosts of ARGs were identified based on the quantitative correlation between these genes and the microbes at the phylum level. Additional statistical analyses including Pearson correlation analysis and one-way ANOVA were performed using R v 3.3.2. Values with $p < 0.05$ or 0.01 were considered statistically significant by SPSS16.0.

3. Results And Discussion

3.1 Sediment Properties

The physicochemical properties, including organic carbon, nitrogen, pH, and phosphorus, have been demonstrated to affect the fate of ARGs in sediments (Zhang et al., 2020b). Therefore, the physicochemical properties of the marine sediments were characterized, which include pH and contents of total carbon (TC), organic carbon (OrgC), ammonium ($\text{NH}_4^+\text{-N}$), nitrate ($\text{NO}_3^-\text{-N}$), total inorganic nitrogen (TIN), and total nitrogen (TN) (Table 1). All the sediments were alkaline, with a pH value ranging from 8.00 to 8.80. The pH of the sediments from the South China Sea was slightly higher than that of the sediments from the other three seas. The TC contents of these sediments were in the range of 8.60 g/kg to 80.43 g/kg. Sediments from the South China Sea had the highest TC content, whereas sediments from the Yellow Sea showed the lowest. The OrgC contents in the sediments ranged from 1.26 g/kg to 32.73 g/kg and presented a similar pattern to the TC. The $\text{NH}_4^+\text{-N}$ contents in sediments from the Bohai Sea (38.43 ~ 67.79 mg/kg) and the Yellow Sea (26.10 ~ 45.65 mg/kg) were much higher than that of the East China Sea (4.84 ~ 26.36 mg/kg) and the South China Sea (7.59 ~ 14.07 mg/kg). On the other hand, there is no apparent difference in the $\text{NO}_3^-\text{-N}$ content in the sediments from different seas, which ranged from 2.37 mg/kg to 7.94 mg/kg. The content of TN in different sediments varied significantly, ranging from 27.42 mg/kg to 315.92 mg/kg.

Table 1

Physicochemical properties of the sediments collected from the Bohai Sea, the Yellow Sea, the East China Sea, and the South China Sea.

Sea area	Sampling sites	Longitude and latitude	pH	NH ₄ ⁺ -	NO ₃ ⁻ -	OrgC	TIN	TN	TC
				N	N				
				(mg/kg)					
The Bohai Sea	NS-25	38.5°N, 119.0°E	8.37	40.34	6.65	14.60	47.00	57.75	18.69
	NS-26	38.5°N, 119.5°E	8.43	67.79	7.94	9.69	75.83	212.44	13.43
	NS-27	38.5°N, 120.0°E	8.26	38.43	5.45	5.21	43.89	93.08	8.60
The Yellow Sea	YS-7	34.0°N, 123.8°E	8.13	45.65	3.05	5.25	48.86	120.82	10.13
	YS-9	34.0°N, 123.0°E	8.00	40.39	2.37	6.48	42.93	220.40	13.12
	YS-11	34.0°N, 122.0°E	8.42	26.10	5.00	1.26	31.20	43.47	11.45
The East China Sea	S01-1	30.0°N, 122.7°E	8.15	4.84	5.54	7.01	12.18	315.92	27.85
	S01-3	30.0°N, 123.5°E	8.22	26.36	6.06	5.50	32.44	126.20	21.01
	S01-5	30.0°N, 124.3°E	8.44	9.78	4.78	4.05	14.60	27.42	15.76
The South China Sea	DZ1	21.8°N, 116.8°E	8.80	9.13	4.01	9.56	13.34	49.51	35.49
	Q02	21.0°N, 118.0°E	8.56	14.07	2.97	32.60	17.17	184.49	80.43
	Q10	20.5°N, 115.5°E	8.70	7.59	3.42	32.73	11.21	57.10	52.14

3.2 Abundance of ARGs and *intI1* in the marine sediments

Due to the wide application of antibiotics β -lactam, sulfonamide, tetracycline, and vancomycin, the corresponding ARGs have been frequently detected in natural environments. The abundances of five prevalent ARGs (i.e. *bla*_{TEM}, *sul1*, *tetA*, *tetC*, and *vanA*) and *intI1* in the marine sediments were determined and presented as relative abundances in Fig. 2. *bla*_{TEM} was the most prevalent and abundant class of ARGs, with a detection frequency of 100% and a relative abundance ranging from 7.6×10^{-5} - 2.7×10^{-3} (Fig. 2) that accounts for 71.5% of the total ARGs (Table S1). Higher abundances of *bla*_{TEM} were found in sediments from the South China Sea, which were in the range of 5.8×10^{-4} - 2.7×10^{-3} . The relative

abundances of *bla*_{TEM} in the Bohai Sea sediments were 1–2 orders of magnitude lower than those in the South China Sea sediments, which ranged from 7.6×10^{-5} to 1.1×10^{-4} . The relative abundances of *bla*_{TEM} in the Yellow sea sediments varied marginally between different sites, ranging from 2.2×10^{-4} – 3.8×10^{-4} . The usage of β -lactams (e.g., cephalosporin and penicillin) were the largest among the four target antibiotics in China (Zhang et al., 2015), which possibly explained the dominance of *bla*_{TEM} in the marine sediments. The usage of sulfonamides in China ranked only second to β -lactams, followed by tetracyclines (Zhang et al., 2015). Consequently, occurrences of *sul1* and *tetC* were also observed in all the sediments, accounting for 19.8% and 7.6% of the total ARGs respectively. The *sul1* genes were more abundant in the sediments of the East China Sea, with a relative abundance of 6.4×10^{-5} – 6.8×10^{-4} . The relative abundances of *sul1* genes were at the minimum in the Yellow Sea sediment, ranging from 4.5×10^{-6} to 3.9×10^{-5} . The relatively higher abundances of *bla*_{TEM} and *sul1* over the other ARGs might be attributed to the wide application of antibiotics from aquaculture (Zhang et al., 2020b). Furthermore, *tetC* genes exhibited higher abundances in the South China Sea and the Yellow Sea, holding relative abundances in the range of 5.8×10^{-6} – 2.4×10^{-4} . *tetA*, another tetracycline resistance gene, was detected with a frequency of 67%. *tetA* was detected with significantly lower relative abundances compared to those of *tetC*, which ranged from 5.9×10^{-7} to 3.8×10^{-5} . The vancomycin resistance gene *vanA* was only detected in the Bohai Sea sediments with a low detection frequency of 25%. The relative abundance of *vanA* was 1–4 orders of magnitude lower than that of the *bla*_{TEM}. Among the five ARGs investigated in this work, *bla*_{TEM} showed the highest abundance in the sediments of four seas from the north to the south of China, with an average of 6.0×10^{-4} . The average relative abundance of *sul1*, *tetA*, *tetC*, and *vanA* in the sediments from four sea sites were 1.6×10^{-4} , 6.7×10^{-6} , 5.7×10^{-5} , and 1.9×10^{-6} respectively. β -lactam, tetracycline, and sulfanilamide were widely used in China. Consequently, the widespread occurrence of *bla*_{TEM}, *tetC*, and *sul1* in various environments has been reported (Fu et al., 2022; Guo et al., 2018; Jia et al., 2018; Leng et al., 2020; Xu et al., 2016).

int1 was detected in all sampling sites excluding Q10 from the South China Sea. *int1* was detected with higher abundances (3.9×10^{-4} to 1.2×10^{-3}) in the East China sea sediments, while those in the South China Sea sediments were relatively lower (0 to 1.0×10^{-4}). In general, the abundance of *int1* demonstrated the tendency of the East China sea > The Bohai sea > the Yellow sea > the South China Sea. The presence of *int1* is beneficial to the dissemination of bacterial resistance (Na et al., 2019), which is used to evaluate the horizontal transfer ability of ARGs. Once ARGs were captured by *int1*, they could be quickly transferred to bacteria (Gillings et al., 2015). ARGs can be further spread via the ocean currents, birds, atmosphere, and animal migration in the environment (Allen et al., 2010).

Taken into all the target ARGs, the relative abundance of total ARGs ranged from 1.0×10^{-4} to 3.2×10^{-3} in the marine sediments. Of the 12 sampling sites, Q10 and Q02 from the South China Sea had higher relative abundances of total ARGs with 3.2×10^{-3} and 1.9×10^{-3} respectively, whereas sediments from the Bohai Sea had the lowest relative abundance of total ARGs ranging from 1.0×10^{-4} to 1.3×10^{-4} . ARGs detected in the South China Sea were more abundant than those in other seas, following the trend

of the South China Sea > the Yellow Sea > the East China Sea > the Bohai Sea. The higher abundance of ARGs in the South China Sea might be attributed to the intensive anthropogenic activities in the Pearl River Delta, which was the dominant source of the ARG contamination. Compared with the occurrence of ARGs in the rivers with a relative abundance up to 2.43×10^{-2} (Wang et al., 2020a), the abundance of ARG in the marine sediments was 1–2 orders of magnitude lower. This was consistent with the ARGs distribution in aquatic environments as rivers receive large volumes of effluents from wastewater treatment plants which were found to contain many ARGs (Zhang et al., 2018a). Although the pollution status in the sea was relatively weaker due to the highly dynamic nature and dilutions of seawater, the ubiquitous presence of ARGs in all sea sediments investigated revealed that seas have suffered from ARGs contamination to some extent.

3.3 Microbial community in the marine sediments

The microbial community of the sediment samples was characterized using 16S rRNA amplicon sequencing. The raw sequencing data of the sediment samples were trimmed and filtered to remove low-quality sequences, generating a total of 1.4 million high-quality reads and an average of 116,834 sequences per sample. The goods coverage of all the sediment samples was greater than 99%, which indicated that the sequencing depth was sufficient to reveal the microbial populations in the sediment samples. The microbial community of the sediment samples was analyzed at the same sequencing depth of 108,782. The relative abundance of phylotypes in the sediments at phylum and genus levels were shown in Fig. 3A and B. At the phylum level, Proteobacteria and Bacteroidetes were the two most abundant phyla in all the sediments (Fig. 3A), with a relative abundance of 21.15%-55.51% and 16.49%-46.89% respectively. Other Phyla such as Firmicutes, Actinobacteria, and Verrucomicrobia were also presented in the sediments with a relatively high abundance of 1.24%~17.98%, 1.89%~5.07%, and 0.27%~13.32% respectively. These five phyla accounted for 73.85 ~ 95.65% of the total population in the sediments. Previous findings have highlighted the abundance of Proteobacteria (comprising > 50% of the microbial biomass) in most surface marine sediments (Dang et al., 2010; Durbin and Teske, 2011; Suzuki et al., 2019). Bacteroidetes were the dominant phylum in sediment samples derived from Cyanobacterial aggregates (Liu et al., 2015b). Proteobacteria and Bacteroidetes, which have been long regarded as dominant microbes in marine sediments and play important roles in the biogeochemical processes (Durbin and Teske, 2011), were predominated in all the sediments samples in this study.

Figure 3B shows the top 10 genera in the sediments, and the genera of *Lutibacter*, *Marinobacter*, *Amphritea*, *Sulfitobacter*, and *Arenibacter* were detected with higher relative abundances. These microbes are distributed globally, many of which have flexible adaptations to various environments of marine sediments (Liu et al., 2015b). The dominant genera of bacteria exhibited distinct spatial distribution. For example, *Lutibacter* were predominant in the Bohai Sea sediments, while *Marinobacter* were detected with higher relative abundances in the Yellow Sea sediments. The genus of *Amphritea* was dominant in the East China Sea sediments, and the genus of *sulfitobacter* was found relatively high in the South China Sea sediments. NMDS analysis of OTUs based on unweighted UniFrac distance matrix was displayed in Fig. 3C. The microbial communities in the sediments from the four seas were clustered into four divergent

groups, suggesting obvious differences in the microbial community between the sea areas. Specifically, sediments from the Bohai Sea (NS-25, NS-26, NS-27), the Yellow Sea (YS-7, YS-9, YS-11), the East China Sea (S01-1, S01-3, S01-5), and the South China Sea (Q02, Q10, DZ1) were divided into group1, group 2, group 3, and group 4 respectively (Fig. 3C). The obvious spatial heterogeneity indicates a correlation between the microbial community and the environment (Liu et al., 2015b). The apparent spatial distribution was further confirmed by UPGMA analysis (Fig. 3D), where sediments from the four seas were categorized into four clusters. Bacterial assemblages of the sediments in the South China Sea appeared to be the most distinct cluster, as these samples were separated into three main clusters. This indicated that different environmental parameters might play important roles in shaping the microbial communities of sediments on the coast of China (Liu et al., 2015b). Similar microbial communities especially key microbial groups might present in environments with significant similarities (Dang et al., 2009).

3.4 Correlation among ARGs, environmental factors, and microorganisms

Environmental factors (e.g., nutrients and organics) have been demonstrated to affect the distribution and abundance of ARGs (Lu et al., 2019b) (Zhang et al., 2020a; Zhao et al., 2019). Therefore, correlation analysis and Mantel test were performed to evaluate the correlations among the target genes, the microbial community, and the environmental factors (Fig. 4A). *bla*_{TEM}, *tetC*, *sul1*, and *int11* were found to be significantly positively correlated with the environmental factors. Specifically, both *bla*_{TEM} and *tetC* showed strong positive correlations with NO₃⁻-N, OrgC, and TC ($p < 0.05$). Positive correlations were found between *sul1* and NH₄⁺-N ($p < 0.05$). In addition, both *bla*_{TEM} and *tetC* were found to be positively related to latitude ($p < 0.05$), while *int11* shown positive correlation with longitude ($p < 0.05$). This study showed that the occurrence of ARGs was positively correlated with the level of nutrients (e.g, nitrate and ammonium) and organics. Therefore, reducing the discharge of conventional pollutants could be an effective way to control the ARG risk in the seas.

Microbial community could be used as a biological indicator of the changes in geochemical and physicochemical characteristics of sediments due to the specificity of microbe habitats (Dang et al., 2010). The correlations between the microbial community and the environmental factors were also demonstrated in Fig. 4A. Proteobacteria were significantly negatively correlated with NO₃⁻-N ($p < 0.05$), whereas Bacteroidetes showed a significant positive correlation with NO₃⁻-N ($p < 0.05$). Bacteroidetes prefer to develop in sediments of eutrophic water environments (Liu et al., 2015b).

Complex correlations were shown between the target genes and the microbial community (Fig. 4A). For instance, a significantly positive correlation was found between the relative abundance of *bla*_{TEM} and Bacteroidetes ($p < 0.05$). Similarly, there was a significant positive correlation between the relative abundance of *sul1* and Acidobacteria ($p < 0.05$). Network analysis was further conducted to reveal the potential host bacteria of the target genes. Colored Nodes represent *int11*, *bla*_{TEM}, *tetC* *sul1*, and potential

host bacteria in Fig. 4B, and connections indicate significant positive or negative correlations ($p < 0.05$). *int1*, *bla*_{TEM}, *tetC*, and *sul1* exhibited strong and significant correlations with 11 bacterial phyla (e.g., Bacteroidetes, Firmicutes, Tenericutes, and Hadesarchaeaeota) ($p < 0.05$), which were speculated to serve as the potential host bacteria for these ARGs. Bacteroidetes and Firmicutes with relatively high abundances (Fig. 3A) were the dominant bacteria host of ARGs in the marine sediments. These bacteria potentially hosted multi-drug resistance, which would develop into antibiotic resistance bacteria through horizontal or vertical gene transfer under ongoing selection pressures of antibiotics (Lu et al., 2019a).

4. Conclusion

Our study demonstrated the occurrence and spatial distribution of ARGs in sea areas of China, from the north to the south. β -lactams resistance genes (*bla*_{TEM}) were ubiquitous and abundant with higher relative abundances up to 2.7×10^{-3} , while vancomycin resistance genes (*vanA*) hold the lowest detection frequency of 25% and the lowest abundances of 5.2×10^{-7} . The South China Sea held the highest abundance of total ARGs, while the Bohai Sea held the lowest of that. Correlation analysis revealed that the distribution of *bla*_{TEM} and *tetC* showed strong correlations with the content of nitrate, organic carbon, and total carbon in sediments, suggesting these factors would affect their occurrence and distribution. Furthermore, the microbial community in the sediments was characterized and the potential host bacteria of ARGs were revealed by network analysis. Bacteroidetes and Firmicutes were shown to be dominant phylum serving as the potential host bacteria for *int1*, *bla*_{TEM}, *tetC*, and *sul1*. This study demonstrated the ubiquitous occurrences of ARGs in the sediments from all seas of China, which encourages effective strategies to control the contamination of antibiotic resistance.

Declarations

Acknowledgments

The authors thank the support of the National Natural Science Foundation of China (No. 52000123 and No. 41907364), Natural Science Foundation of Jiangsu Province (No. BK20200816), the Natural Science Foundation of Shandong Province (No. ZR2020QE233 and No. ZR2021MD037), the NSFC Shiptime Sharing Project (No. 41949905), and the Major Scientific and Technological Innovation Project of Shandong Province, China (No. 2021CXGC011206).

Authors' contributions

Shuai Zhang and Shuai Liu were responsible for the experiment design. Hui Chen performed the experiments and drafted the manuscript. Minggang Zheng was responsible for the sediments sampling. Weijian Liu carried out the data processing. Shaoping Kuang and Minge Tian revised the manuscript. All the authors read and approved the version of the manuscript.

Ethics Approval

Not applicable

Consent to Participate

Not applicable

Consent for Publication

Not applicable

Conflict of Interest

The authors declare no competing interests.

Data availability

Not applicable

Code Availability

Not applicable

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Figures

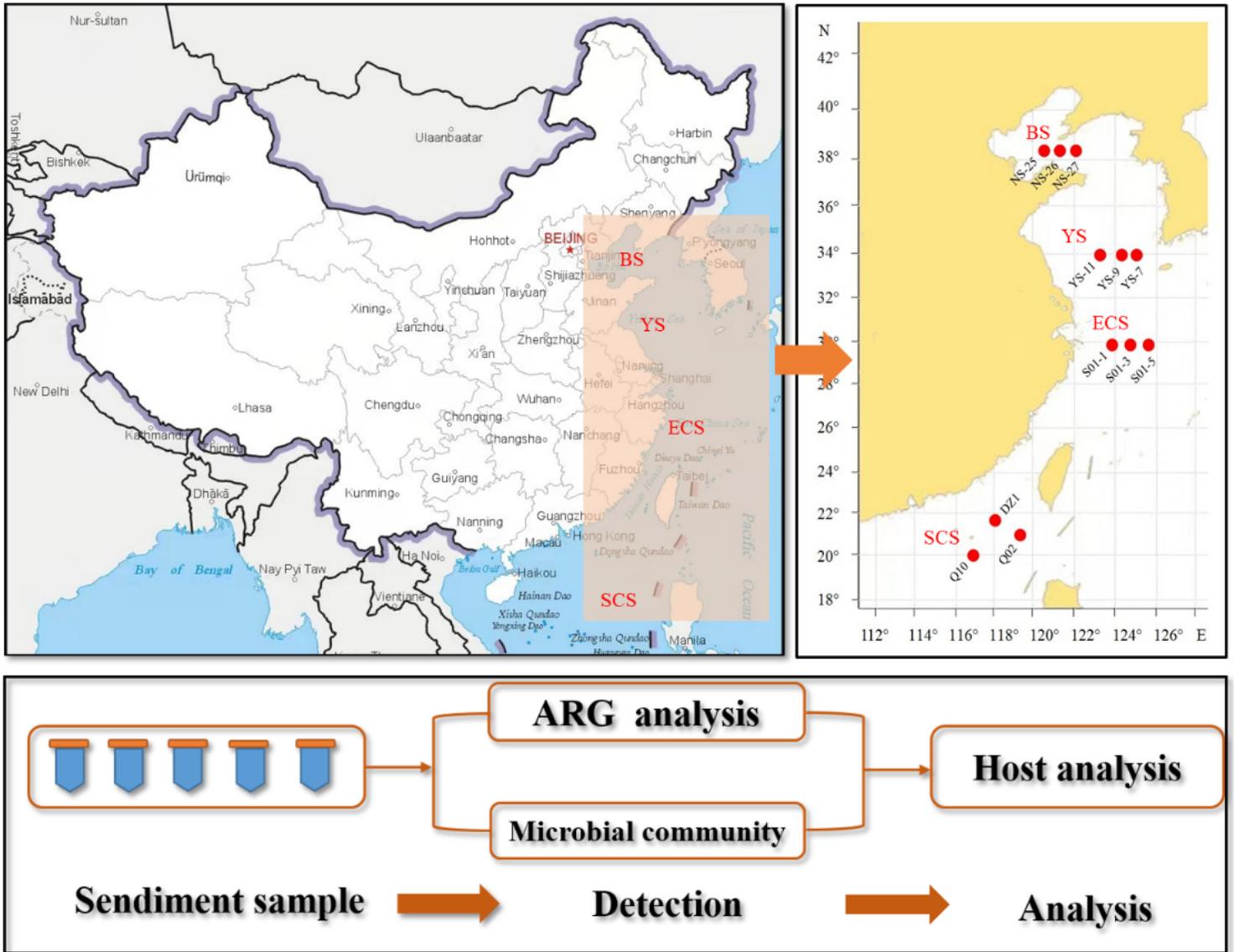


Figure 1

Study areas and sediment sampling sites in the Bohai Sea, the Yellow Sea, the East China Sea, and the South China Sea.

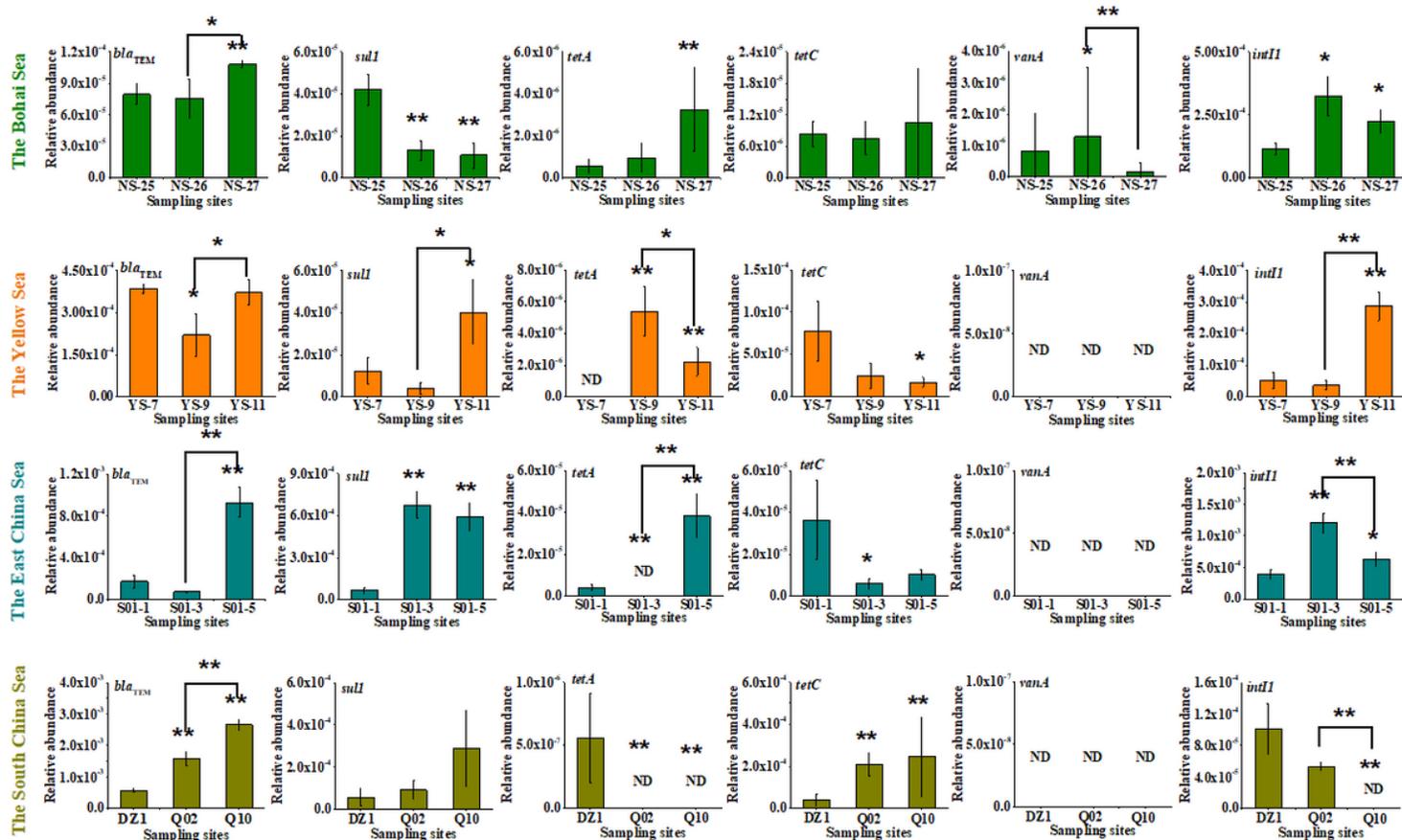


Figure 2

The relative abundance of *bla_{TEM}*, *sulI*, *tetA*, *tetC*, *vanA*, and *intI1* in the sediments from the Bohai Sea, the Yellow Sea, the East China Sea, and the South China Sea.

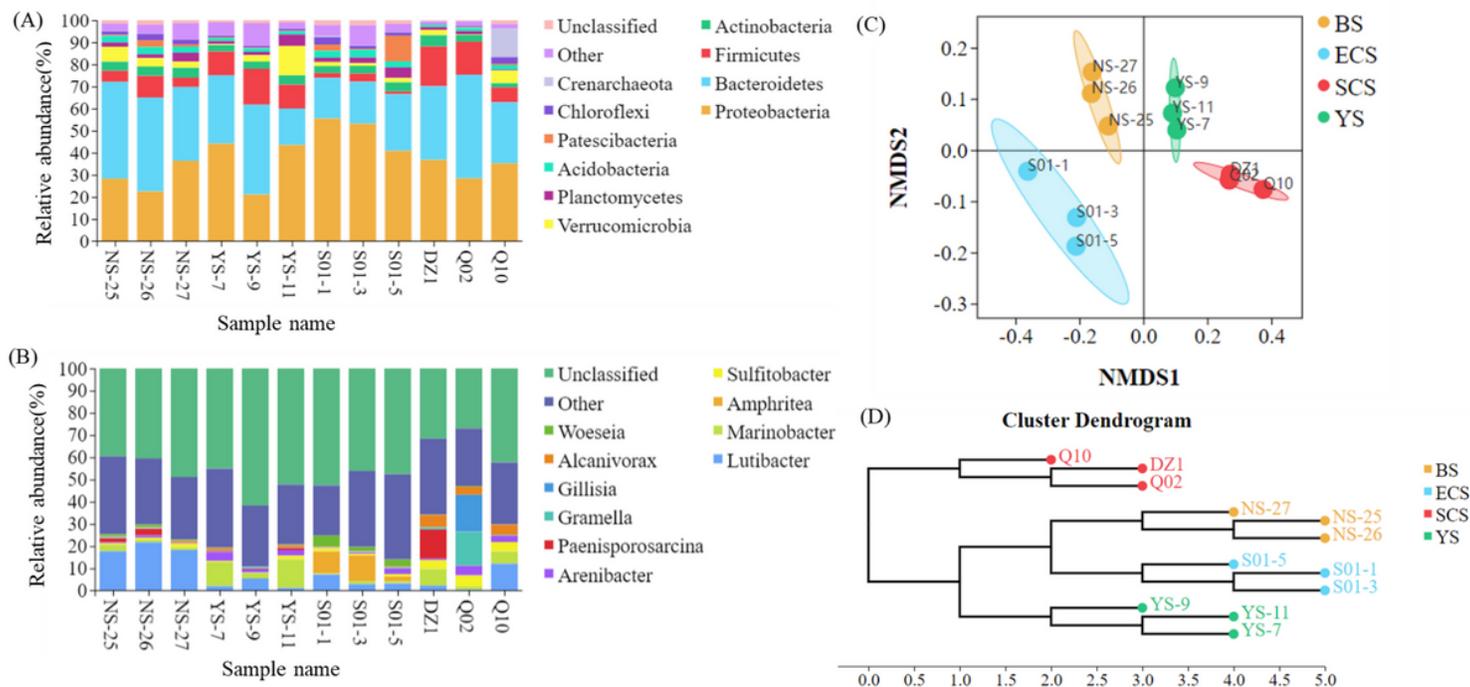


Figure 3

Microbial community of the marine sediments. (A) Composition of microbial population at phylum level, (B) Composition of microbial population at genera level, (C) NMDS analysis plot of marine sediments based on the unweighted UniFrac distance matrix, (D) UPGMA clustering tree of the marine sediments. Only the top 10 phyla or genera were presented, while the remaining sequences were classified as 'Other' and 'Unclassified'.

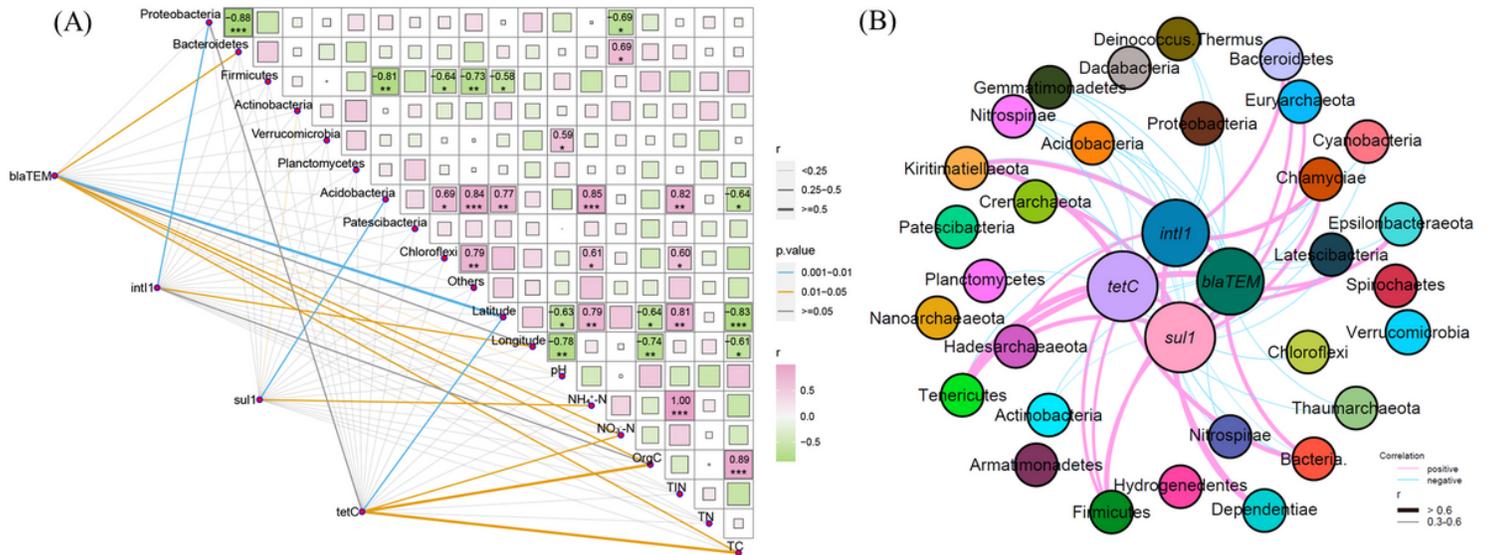


Figure 4

(A) Correlation analysis (upper right diagram) and Mantel test (bottom left diagram) show the correlations among ARGs, microbes, and environmental factors in the marine sediments. Significant correlations with an p -value of < 0.05 , < 0.01 , and < 0.001 were denoted using *, **, and *** respectively. (B) Network analysis displays the correlation between target genes and the potential host microorganisms.

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