ANTHROPOGENIC INFLUENCES ON COASTAL BACTERIOMES AND ANTIBIOTIC RESISTOMES: FROM ENVIRONMENT TO CULTURED FISH

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Anthropogenic Influences on Coastal Bacteriomes and Antibiotic Resistomes: From Environment to Cultured Fish

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Certificate of Originality

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_____(Signed)

____Zhang Xiaohua____(Name of student)

Abstract

Anthropogenic influences on coastal bacteriomes and resistomes can promote the proliferation of human pathogens and antibiotic resistance genes (ARGs) in coastal environments and their dissemination into marine organisms. These impacts are likely amplified in intensively managed mariculture systems, raising critical concerns about seafood safety. However, the extent and mechanisms of anthropogenic influences on coastal bacteriomes and resistomes, as well as the resulting seafood contamination, remain poorly characterized, particularly in mariculture contexts. This poses significant threats to environmental and human health. To address these issues, we provide metagenomic insights into alterations in the characteristics of coastal bacteriomes and resistomes under varying anthropogenic influence across different seasons in two megacities, using minimally impacted oceanic ecosystems as global baselines, and identified key drivers. Then, we assessed the link between environmental alterations and the emergence of human pathogens and antimicrobial resistance at a mariculture site using metagenomic and culture-based methods.

Relative to oceanic baselines, coastal bacteriomes showed a marked decline in biodiversity, a significant rise in human pathogen abundance, and a notable shift in core bacterial species, while coastal resistomes exhibited greater diversity, abundance, mobility, and co-occurrence with human pathogens. These biological groups showed more obvious seasonal variations than regional fluctuations from diversity to composition. Bacterial diversity peaked in winter, accompanied by a decline in human

pathogen abundance. The diversity, abundance, mobility, and host range of ARGs were elevated in winter, particularly in Hong Kong coastal waters. These patterns involved increased levels of human-associated bacteria and ARGs, underscoring the profound influences of anthropogenic activities on coastal bacteriomes and resistomes.

Mechanistic analyses revealed that coastal bacteriomes and resistomes were shaped by a combination of environmental and anthropogenic factors. However, biodiversity loss, shifts in core bacterial species, human pathogen proliferation, and resistome emergence were more influenced by anthropogenic factors, including antibiotic selection pressure, human fecal contamination, and anthropogenically enhanced ARG transfer. The elevated stress from these factors during winter, particularly in Hong Kong coastal waters, largely contributed to the proliferation of human-associated bacteria and ARGs in coastal waters, thereby driving the adverse shifts in costal bacteriomes and resistomes. Wastewater treatment plant discharge and contaminated riverine runoff were primary origins of these drivers. Critically, these alterations in coastal bacteriomes and resistomes have amplified health risks for coastal populations through increasing exposure to multidrug-resistant human pathogens.

Further metagenomic investigations into the link between environmental alterations and seafood contamination at the contaminated mariculture site unveiled that mariculture-induced environmental changes promoted the enrichment of emerging and foodborne pathogens (e.g., *Staphylococcus aureus*, *Klebsiella pneumoniae*, and

Escherichia coli) and typical ARGs conferring resistance to vancomycin, macrolide–lincosamide–streptogramin antibiotics, trimethoprim, and chloramphenicol in cultured fishes. Shifts in source dynamics and the enhancement of ARG transferability further amplified this enrichment. The isolation of methicillin-resistant *S. aureus* in edible fish tissues provides phenotypic evidence for these findings and highlights the health risks of processing and consuming such contaminated seafood.

Collectively, this systematic study offers comparatively systematic insights into the anthropogenic impacts on coastal bacteriomes and resistomes in both environmental and cultured seafood contexts. The findings advance our understanding of bacterial and ARG contamination and its associated health risks to coastal ecosystems and resident communities, ultimately supporting the urgency of developing effective strategies to address the growing threats of coastal antimicrobial resistance and pathogen proliferation at the local and global scales.

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Chater 1 Introduction

1.1 Background

Bacteriomes and resistomes are intrinsic components of natural microbiomes in pristine ecosystems, where they exhibit dynamic adaptions to environmental conditions without human influence (Ahlstrom et al., 2018; Belkin & Colwell, 2006; D'Costa et al., 2011; Gutierrez West et al., 2013; Van Goethem et al., 2018). However, anthropogenic activities have profoundly disrupted these microbial components by introducing substantial quantities of human-associated chemical and biological agents. Such disturbances have catalyzed their shifts and the resultant prevalence of human pathogens and antimicrobial resistance in recent decades. This trend has led to a concerning escalation in bacterial pathogenicity, presenting a critical global challenge with profound consequences for ecological stability and public health (O'Neill, 2016; Smith et al., 2014). In response, extensive research has sought to characterize the anthropogenic impacts on bacteriomes and resistomes across diverse human-associated environments, while also elucidating the underlying mechanisms driving these changes (Arnone & Perdek Walling, 2006; Cui et al., 2019; Fang et al., 2019; Kang et al., 2022; Obayomi et al., 2019; Pitta et al., 2016; Reddy et al., 2022; Wang et al., 2023; Yang et al., 2016).

Marine coastal ecosystems near densely populated urban centers are increasingly subject to multifaceted stressors arising from anthropogenic activities. Recent studies have revealed significant bacterial and antibiotic resistance gene (ARG) contamination

in human-impacted zones, including wastewater treatment plant (WWTP) discharge sites, estuaries, mariculture areas, and recreational waters (Fresia et al., 2019; Gabashvili et al., 2022; He et al., 2023; Jeffries et al., 2016; Kvesić et al., 2022; Leonard et al., 2022; Sala-Comorera et al., 2021; Zhou et al., 2022). Although anthropogenic contributions to such contamination are well recognized, the extent and mechanisms of human impacts on coastal bacteriomes and resistomes remain largely unclear. These critical knowledge gaps highlight the pressing need for a comprehensive investigation to elucidate the characteristics and underlying drivers of coastal microbial ecosystems under varying anthropogenic influence relative to pristine baselines.

Anthropogenic alterations in coastal bacteriomes and resistomes may profoundly affect marine animal microbiomes, raising significant concerns about seafood safety. This is especially critical in mariculture, a major source of global seafood production, where extensive antimicrobial and feed use has driven the proliferation of human pathogens and antimicrobial resistance in environments and cultured organisms (Reverter et al., 2020; Schar et al., 2021). Cultured seafood has been identified as reservoirs for these hazardous elements, facilitating their transmission through marine ecosystems to human populations and posing substantial public health risks (Heuer et al., 2009; Santos & Ramos, 2018). However, the mechanisms linking mariculture-induced environmental changes to seafood contamination remain poorly characterized. Moreover, there is increasing concern over the potential link between seafood contamination and global outbreaks of community-based diseases, yet this relationship

has received limited research attention. These knowledge gaps challenge sustainable management strategies, effective contamination mitigation, and high-quality seafood production in coastal ecosystems. Comprehensive research is urgently needed to unveil the underlying mechanisms and health risks of biological contamination of cultured seafood associated with mariculture practices.

Collectively, to mitigate the microbial contamination in coastal environments and cultured seafood, it is necessary to conduct a holistic investigation into the extent, mechanistic drivers, and health consequences of anthropogenic impacts on bacteriomes and resistomes in these ecosystems. The insights obtained from such research will provide a critical evidence base for regulatory agencies in coastal regions, enabling the development of targeted strategies to safeguard both environmental integrity and public health within the integrative One Health framework.

1.2 Research objectives

This thesis aims to advance our understanding of anthropogenic influences on the bacteriomes and resistomes in coastal waters and in cultured seafood, as well as the resultant health risks via waterborne and foodborne pathways. To achieve these goals, a seasonal sampling campaign was conducted to collect surface seawater samples from coastal waters affected by diverse anthropogenic activities (e.g., sewage discharge, mariculture, transportation, recreation, estuary, reservation) in two well-urbanized megacities along China's southern and northern coastlines, alongside seafood collection

in a representative mariculture farm in Hong Kong's fish culture zones. The minimally impacted surface oceanic systems and wild-caught fishes in Hong Kong coastal waters were employed as a baseline for comparison. This project tackles the proposed scientific questions by using the research framework shown in Figure 1-1. The major objectives are:

1) To delineate distinctions in the structure and variations of bacteriomes and resistomes between coastal and oceanic environments; and

2) To explore the drivers and health risks of coastal microbial alterations; and

3) To elucidate mariculture-induced biological contamination of cultured seafood.

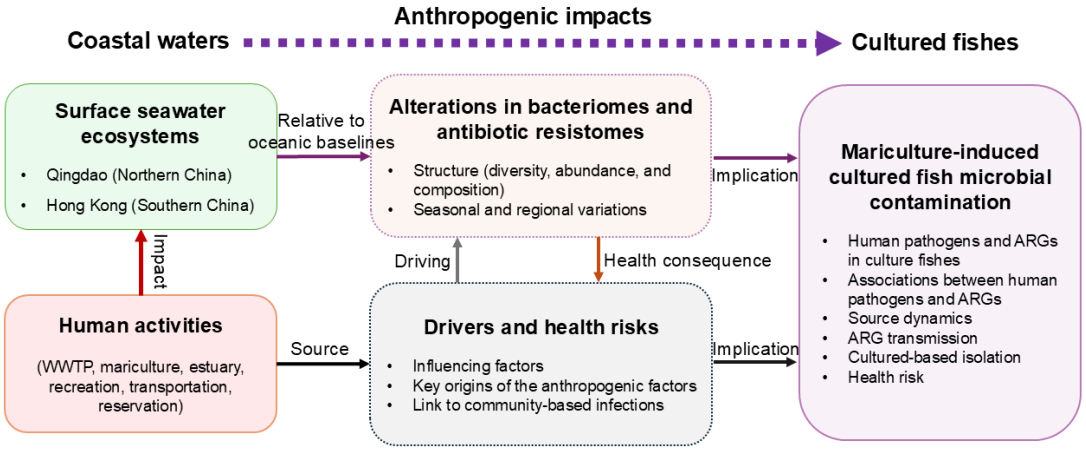

Figure 1-1 Research framework of this PhD study.

1.3 Organization

The first chapter introduces the background information of bacteriomes and resistomes in human-impacted systems, states the existing knowledge gaps and the necessity to investigate baseline-related alterations of these microbial components in coastal environments and cultured seafood, and finally presents the aims and key objectives of

the research. Chapter 2 reviews the current research and clarifies the knowledge gaps related to bacteriomes and resistomes in coastal waters and cultured fishes. Chapter 3 provides detailed information about the cities studied, sampling campaigns, experimental and bioinformatics strategies, and statistical methods. The subsequent three chapters discuss the primary findings of the research. Chapter 4 pinpoints anthropogenic alterations in the structure and variations of coastal bacteriomes and resistomes compared with the baselines. As an extension of Chapter 4, Chapter 5 elucidates the drivers and health risks of microbial alterations. In response to the observed rise of harmful elements in mariculture zones in Chapter 5 and the concerns for seafood safety, Chapter 6 explores the emergence of these elements in cultured fishes, quantifies their source contributions, estimates the influence of farming practices on the environmental rise of these elements and its association with seafood contamination, and provides advice on contamination mitigation. Finally, Chapter 7 concludes with the main findings and limitations of the current research and offers recommendations for potential future study.

Chater 2 Literature review

Bacteriomes and resistomes are essential constituents of microbial communities in natural ecosystems, where they play a crucial role in preserving ecological stability. Nevertheless, these components are increasingly perturbed by anthropogenic activities, particularly in coastal regions. This chapter provides a comprehensive review of previous studies on the structural and variations of bacteriomes and resistomes in coastal waters affected by human activities, with a specific emphasis on the emergence of human pathogens and antimicrobial resistance in cultured seafood. By synthesizing the current research, this review identifies critical knowledge gaps and proposes future research directions to address the urgent challenges posed by microbial shifts in coastal ecosystems.

2.1 General concepts of bacteriomes and resistomes

2.1.1 Bacteriomes and resistomes in natural habitats

Bacteriomes represent the major component of individual microbiomes, characterized by diverse prokaryotic populations and their interactions, as well as dynamic alterations over time driven either by natural processes or by multiple external factors (Patrinos et al., 2020). As a fundamental component of microbial ecosystems, they are critical for maintaining host health and ecological stability. Representing one of the most abundant and biodiverse groups of organisms (Horner-Devine et al., 2004), bacteriomes are composed of 52 recognized bacterial phyla, dominated by *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Acidobacteria*, *Cyanobacteria*, and

Verrucomicrobia (Shin et al., 2015). The relative abundance of these groups varies across habitats, reflecting their adaptation to distinct environmental niches. While pathogenic bacteria are part of these communities, they generally occur at low concentrations and exhibit limited virulence in stable ecosystems due to the regulatory effects of biodiversity and ecological resilience (Craft et al., 2022). Key environmental variables, including temperature, nutrient availability, pH, and salinity, play a pivotal role in shaping bacterial community composition and driving evolutionary trajectories. Consequently, bacterial biodiversity and composition exhibit pronounced spatial-temporal patterns, reflecting seasonal and regional variations in these factors (Haggerty & Dinsdale, 2017; Malard et al., 2019).

Antibiotic resistomes, comprising a diverse array of genes that confer antibiotic resistance, represent a natural evolutionary adaptation and self-protection mechanism for microorganisms. These genes arise in response to competitive pressures and selective forces exerted by antibiotics produced by other microbes in dynamic environments (Martinez et al., 2009). Resistome acquisition occurs through mutations (Martinez & Baquero, 2000) or horizontal gene transfer (HGT) facilitated by mobile genetic elements (MGEs) (Frost et al., 2005). Despite their current prominence in anthropogenically impacted settings, resistomes are intrinsic to pristine ecosystems and have been detected in ancient and remote environments, such as 30,000-year-old Beringian permafrost sediments (D'Costa et al., 2011) and 3,000-m deep-sea sediments (Chen et al., 2013). This ubiquity underscores their long-standing role in microbial

ecology and evolution.

2.1.2 Bacteriomes and resistomes in human-associated environments

Due to the significant selective pressures introduced by human activities, such as the release of vast quantities of chemical and biological agents (e.g., antibiotics and human-associated bacteria), the structure and variations of these natural elements have been profoundly altered by anthropogenic influences. Human-associated pathogens from phyla, *Proteobacteria* and *Bacteroidetes,* are increasingly prevalent in human-impacted environments, marked as microbial dysbiosis (Holcomb & Stewart, 2020; Niepceron et al., 2013). Concurrently, the rise of antimicrobial resistance is exacerbated by the misuse and overuse of antibiotics in medical and veterinary contexts (Ferri et al., 2017). The proliferation of resistomes and antibiotic-resistant pathogens is now widespread across diverse human-impacted systems, including urban environments (Arnone & Perdek Walling, 2006; Cui et al., 2019; Reddy et al., 2022; Zhang et al., 2022), agricultural soils (Han et al., 2018; Li et al., 2020; Obayomi et al., 2019; Pitta et al., 2016; Wang et al., 2023), animal farms (Kang et al., 2022; Yang et al., 2016), and aquaculture compartments (Cui et al., 2022; Fang et al., 2019; He et al., 2023). Notably, seasonal or regional trends in bacteriomes and resistomes have also been identified in some of these settings (Geremia et al., 2016; Liu et al., 2018; Xiong et al., 2014).

With the emergence of the above-mentioned harmful elements, the sustainability of economic and human health systems faces significant challenges. Since the 1980s, the

incidence of disease outbreaks caused by pathogenic bacteria has steadily increased, culminating in a mortality burden of at least 7.7 million deaths globally in 2019 (Ikuta et al., 2022; Smith et al., 2014). Among the leading contributors to this crisis are *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, collectively responsible for significant mortality worldwide (Ikuta et al., 2022). Projections from the landmark review on antimicrobial resistance have estimated that without immediate intervention, drug-resistant infections could claim 10 million lives annually and result in a cumulative economic loss of $100 trillion by 2050 (O'Neill, 2016). These trends underscore the urgent need for global actions to monitor the prevalence of these harmful elements in human-related environments and clarify their anthropogenic mechanisms for contamination management.

2.2 Bacteriomes and resistomes in human-impacted coastal waters

Marine coastal ecosystems are highly impacted by humans and receive a huge amount of chemical and bacterial contaminants (e.g., heavy metals, antibiotics, human-derived bacteria, and other genetic elements) from sewage discharge, recreational activities, transportation, mariculture, riverine runoff, ecological tourism, and so on (Shahidul Islam & Tanaka, 2004). Shifts in the structure and variations of bacterial communities and the rise of human pathogens and resistomes have been documented in these human-impacted coastal ecosystems (Nogales et al., 2011).

2.2.1 Characteristics of coastal bacteriomes

2.2.1.1 Bacterial community composition

Many anthropogenically impacted coastal waters, such as WWTP discharge sites, estuaries, mariculture areas, and recreational waters, have shown a significant shift in bacterial communities compared with coastal control sites (Cury et al., 2011; Nogales et al., 2011; Won et al., 2017). Dominant bacterial phyla display significant variations in abundance depending on specific human activities. For example, *Proteobacteria* accounts for 99%, 23.40%–59.3%, and 20.1%–87.1% of bacterial communities in sand mining sites (Won et al., 2017), submarine effluent-receiving coastal waters (Kvesić et al., 2022), and mariculture systems (He et al., 2023), respectively. *Firmicutes* shows a marked increase in water zones affected by WWTP effluents compared with other human-impacted regions (He et al., 2023; Kvesić et al., 2022; Won et al., 2017). Moreover, a significant increase in the relative abundance of contamination-adapted bacterial species has been observed in bacterial communities of coastal waters. Polycyclic aromatic hydrocarbon (PAH)-degrading bacteria are reported to be enriched in PAH-contaminated harbors, highlighting the specificity of bacterial responses to contaminants, which differ markedly from those observed in wastewater-affected areas (Sauret et al., 2016). Recently, these findings have been supported by the significant heterogeneity in bacterial composition and dominant taxa across regions subject to varying levels of human activities using advanced sequencing techniques (Fresia et al., 2019; He et al., 2023). These observations suggest that shifts in coastal bacterial community structure may be closely linked to the intensity of human activities and the

concentration of selection agents in these systems. These stressors may promote the emergence of contamination-selected bacterial species, leading to significant alterations in community composition.

Previous investigations on global ocean bacteriomes have demonstrated that bacterial community composition exhibits marked spatial-temporal variability, with seasonal fluctuations often surpassing spatial differences in magnitude (Castillo et al., 2022; Fuhrman et al., 2015; Sunagawa et al., 2015). These patterns reflect the intrinsic variations of marine microbial ecosystems, shaped by environmental heterogeneity driven by factors such as temperature, salinity, the chlorophyll *a* concentration, photoperiod, and nutrient availability (Castillo et al., 2022; Fuhrman et al., 2015; Sunagawa et al., 2015). However, bacterial community composition in anthropogenically impacted regions has exhibited irregular spatial and seasonal variations that deviate from the patterns observed in less disturbed systems (Nogales et al., 2007). This highlights the natural heterogeneity in coastal systems that may be disturbed by human activities. To better understand this issue, more research is essential to uncover the role of anthropogenic impacts in these alterations.

2.2.1.2 Biodiversity and human pathogens

Recent findings have revealed that bacterial diversity often decreases in anthropogenically influenced coastal systems (Abreu et al., 2022; Reuver et al., 2022). Such decline is related to the suppression of ecologically valuable and diverse bacterial

species in microbial communities due to the influx of chemical compounds, which reach the toxic level (Chakraborty & Bhadury, 2015). Meanwhile, human-associated pathogens are increasingly prevalent in anthropogenically impacted coastal waters due to their introduction via wastewater discharge, riverine input, recreational activities, and mariculture farming (Blaak et al., 2014; Fresia et al., 2019; Gabashvili et al., 2022; Jo et al., 2021; Zhou et al., 2022). Pathogen abundance increases with higher levels of anthropogenic influence (Jurelevicius et al., 2021). Currently, the coastal pathogen pool consists of indigenous and introduced species, with a large proportion classified as *Gammaproteobacteria* (Thompson et al., 2005). Anthropogenically introduced pathogens, such as *K. pneumoniae*, *E. coli*, *S. aureus*, and *Salmonella,* exhibit high concentrations in the above-mentioned coastal zones impacted by wastewater discharge, riverine input, recreational activities, and mariculture farming (Blaak et al., 2014; Fresia et al., 2019; Gabashvili et al., 2022; Jo et al., 2021; Zhou et al., 2022).

Previous investigations have demonstrated regional and seasonal variability of bacterial biodiversity in coastal and oceanic ecosystems, with this microbial index peaking in winter (Gilbert et al., 2012; He et al., 2017; Jeffries et al., 2016; Meziti et al., 2015; Sunagawa et al., 2015). However, this variability is being altered by human activities in coastal systems. The regional heterogeneity of biodiversity in some human-impacted areas has been observed to exhibit a close relationship with the variations in the levels of anthropogenic influences (He et al., 2017). Human pathogens often proliferate in warm water temperatures and reach a seasonal peak in abundance during warmer

months in natural marine environments (Belkin & Colwell, 2006). This pattern further implies an increase in pathogen prevalence in warmer regions. Such trends are also observed in human-impacted coastal systems. For example, the seasonal variability in enteric pathogen abundance is linked to temperature changes in subtropical estuaries (Lipp et al., 2001). *Vibrio* communities show a pronounced seasonal increase during summer compared with winter (Böer et al., 2013; Oberbeckmann et al., 2012). These observations underscore temperature as a crucial driver of pathogen seasonality in both natural and human-impacted marine environments. However, a recent study has reported that the spatial and seasonal variations in pathogen abundance and composition along the coast of the Visakhapatnam region are attributed to the influx of human-derived pathogens rather than temperature (Behera et al., 2023). This suggests that pathogen loading may become more critical than temperature for the regional and seasonal distributions of human pathogens in highly human-impacted coastal waters. To further clarify this hypothesis, in-depth research on the variations of potential pathogenic communities is imperative. Additionally, human health loss is closely related to the proliferation of introduced pathogens, given their established role in causing illnesses through various transmission routes, including seafood consumption, direct seawater exposure, and marine zoonoses (Thompson et al., 2005). As human reliance on coastal environments intensifies, a comprehensive investigation is urgently needed to clarify the extent of pathogen emergence.

2.2.2 Characteristics of coastal resistomes

2.2.2.1 Diversity, abundance, and profile

Resistomes are naturally occurring and exhibit low concentrations in pristine environments. However, significantly higher diversity and abundance of resistomes have been documented in human-impacted coastal zones compared with less-impacted control sites. For example, diverse ARGs are found in mariculture environments along the Chinese coastlines (He et al., 2023), while the Pearl River estuaries in China, surrounded by large urban cities, harbor greater ARG diversity and abundance than estuaries in less urbanized regions (Zhou et al., 2022). The level and profile of these biological elements also exhibit substantial heterogeneity, varying with the intensity of surrounding anthropogenic activities (Fresia et al., 2019; Jurelevicius et al., 2021). These variations are reported to be closely linked to the diversity and concentration of antibiotics introduced through human activities (Fresia et al., 2019; He et al., 2022; Zheng et al., 2021). Culture-based or metagenomic studies have revealed that ARGs encoding resistance to key prescription human and animal medicine classes – such as fluoroquinolones, tetracyclines, sulfonamides, macrolides, beta-lactams, aminoglycosides, and streptogramins – are more dominant in anthropogenically impacted areas, such as WWTP discharge zones, estuaries, recreational waters, and mariculture areas, compared with less-impacted sites (Blaak et al., 2014; Fresia et al., 2019; Gabashvili et al., 2022; He et al., 2023; Jo et al., 2021; Zhou et al., 2022). These findings highlight the influence of anthropogenic activities on the rise of resistomes in human-impacted coastal waters.

A recent study on antimicrobial resistance in marine systems revealed significant biogeographic differences in the mean abundance of oceanic ARG classes (Cuadrat et al., 2020). Although the authors of that study did not explore seasonal variations, the authors of another investigation linking oceanic gene functional composition to surface seawater temperature suggested that pristine marine resistomes may exhibit temporal variability (Sunagawa et al., 2015). Accordingly, it is likely that marine resistomes exhibit both regional and seasonal variability in nature. However, recent years have seen clear evidence of anthropogenic disturbances on the regional or seasonal distributions of ARG diversity and abundance in various coastal waters, including the recreational zones (Makkaew et al., 2021; Shi et al., 2023), the coastlines and estuaries of China (Lu et al., 2020; Zheng et al., 2022), and the estuaries of Mandovi and Zuari (Toraskar et al., 2022). Contrasting patterns have been observed across these impacted regions. For example, ARG abundance and diversity in China's coastal waters peak during winter (Lu et al., 2020), whereas the Yangtze River Estuary exhibits higher levels in summer (Liu et al., 2023). These discrepancies underscore the complex interplay between localized anthropogenic pressures and environmental drivers, yet they also highlight gaps in our understanding of ARG dynamics. Given that the accumulating human influence could fundamentally alter the intrinsic regional and seasonal features of ARGs in coastal systems, there is an urgent need for high-resolution studies to elucidate these effects and inform effective management strategies.

2.2.2.2 MGEs and ARG transmission

Naturally occurring ARGs typically exhibit low mobility. In contrast, human-associated ARGs are highly prone to rapid dissemination among environmental bacteria and transfer to clinically relevant pathogens via HGT under selective pressure (Carr et al., 2021; Castañeda-Barba et al., 2024). This process plays a pivotal role in the proliferation of antimicrobial resistance, influencing the dynamics of host bacteria and resistant pathogens within bacterial communities (Larsson & Flach, 2022). A variety of MGEs, including plasmids, transposases, bacteriophages, integrons, and recombinases, have been identified as key vectors for ARG transfer (Carr et al., 2021).

Historically, MGEs involved in ARG transmission have been well characterized in human-influenced terrestrial and freshwater environments, such as agricultural soils, manure, wastewater, and rivers (Castañeda-Barba et al., 2024; Karkman et al., 2018; Lee et al., 2020; Lima et al., 2020; Wu et al., 2023). Recently, their investigations have extended into coastal ecosystems. The presence of MGEs, including integrons (*intI1*, *intI2*, and *intI3*), insertion sequences (*IS91*), transposons (*tnpA*), and plasmids, have been found in estuaries, WWTP discharge areas, beaches, and coastal aquaculture zones (An et al., 2023; Fresia et al., 2019; Zhou et al., 2022). In these settings, ARGs are often positively associated with or co-located on these MGEs, suggesting that MGEs are widespread in human-impacted coastal waters and may play a critical role in facilitating ARG transmission. On the other hand, human-associated ARGs conferring resistance to sulfonamides, beta-lactams, aminoglycosides, and macrolides are observed to be

frequently carried on plasmids and widely distributed among clinically significant enterobacteria in the receiving waters of WWTP sewage, including *E. coli*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Acinetobacter* (Fresia et al., 2019). These observations underscore the importance of MGEs in the acquisition and spread of antimicrobial resistance among coastal bacteria and human pathogens under anthropogenic stress.

Several studies have reported HGT events involving core ARGs and their associated hosts in oceanic environments (Cuadrat et al., 2020; Xu et al., 2023; Yang et al., 2022). However, the spatial-temporal dynamics of this process across global oceans remain inadequately explored. In recent years, the co-occurrence of ARGs and MGEs has become increasingly evident in human-impacted coastal environments (Lu et al., 2020; Peng et al., 2024). These elements exhibit a similar seasonal increase during winter and show a significant correlation with one another (Peng et al., 2024). These observations suggest that anthropogenic pressures may foster a stronger association between resistomes and mobilomes, which could, in turn, disrupt the natural variations of ARG transfer. This altered relationship could facilitate the spread of ARGs to host bacteria, thereby influencing the distribution patterns of resistant bacteria and pathogens. Despite the growing recognition of these processes, comprehensive studies investigating the mobilomes and their resultant regional and seasonal variations of ARG transfer in both pristine and human-impacted marine ecosystems remain scarce. Further research is essential to tackle this important issue.

2.2.2.3 Antibiotic-resistant bacteria (ARB) and human pathogens

The increasing prevalence of antibiotics, antimicrobial resistance, and MGEs has facilitated the acquisition of resistance by bacteria and human pathogens in anthropogenically impacted coastal waters through HGT and mutations under selective pressures. This has led to the emergence of antibiotic-resistant species. Various ARB and human pathogens have been detected in these waters, particularly in the zones impacted by WWTP effluents, riverine runoff, and mariculture, including emerging and foodborne pathogens, such as *E. coli* (Blaak et al., 2014), *S. aureus* (Akanbi et al., 2017), *Pseudomonas* spp. (Luczkiewicz et al., 2015), and *Vibrio* spp. (Kumarage et al., 2022). These bacteria commonly exhibit resistance to antibiotics used in healthcare and agriculture, such as beta-lactams, tetracyclines, aminoglycosides, macrolides, and quinolones. The high concentrations of these antimicrobials, as well as the enhanced transferability of ARGs, are likely contributing factors to the increased abundance of these biological agents in affected coastal zones (He et al., 2022; Zheng et al., 2021). Such proliferation of ARB and resistant pathogens has been observed to be more pronounced with the increasing intensity of anthropogenic influences. A recent study highlighted that sewage-impacted environments harbor a greater diversity of bacterial genera and human pathogens carrying mobile ARGs than beach environments (Fresia et al., 2019). Despite these insights, significant gaps remain in understanding the structural diversity and spatial-temporal variations of ARB and resistant pathogens, as well as their driving factors.

2.3 Influences of human activities on coastal bacteriomes and resistomes

2.3.1 Impacts of different anthropogenic activities

As discussed above, many studies have indicated the impacts of anthropogenic activities on coastal bacteriomes and resistomes. Different structures and variations of bacteriomes and resistomes have been observed in coastal waters under distinct anthropogenic stresses, resulting from the differential inputs of chemical and biological contaminants. WWTP discharge, riverine input, and mariculture farming seem to be more important in driving the alterations of coastal bacteriomes and resistomes than other anthropogenic activities given that their impacted zones show a significantly higher divergence of these biological elements from the control sites compared with other human-impacted areas. Despite these insights, much of the existing research has focused on a single human activity or a narrow range of human activities, impeding a holistic assessment of the relative contributions of these diverse stressors and limiting the accuracy of these observations. On the other hand, the absence of baseline data in these studies has led to an underestimation of the full extent of anthropogenic impacts on natural bacterial communities and makes it difficult to compare and synthesize the results of multiple studies that employ different control sites located in coastal areas.

While recent studies have identified riverine runoff, sewage discharge, and aquaculture as the primary contributors to the prevalence of human pathogens and ARG pollution in estuarine and coastal environments, these efforts remain insufficient due to their limitation in the scope of antibiotic resistance subtypes and pathogenic species (Stewart

et al., 2008; Zheng et al., 2021). The data used primarily derives from traditional techniques (methods based on culturing and quantitative real-time polymerase chain reaction [qPCR] methods), which are inadequate to comprehensively capture the genetic elements in samples. In fact, the authors of one review identified only 23 prevalent resistome subtypes across global coastal waters – significantly fewer than those uncovered by advanced next-generation sequencing (NGS) and computational methods (Blaak et al., 2014; Fresia et al., 2019; Gabashvili et al., 2022; He et al., 2023; Jo et al., 2021; Zhou et al., 2022). With the advancements in sequencing technologies and bioinformatics analysis, current metagenomic approaches can uncover unprecedented genetic information within a sample, shedding light on the comprehensive surveillance of biological elements and their contributions from anthropogenic activities. Moreover, recent metagenomic datasets from a global investigation of microbiomes in relatively pristine oceanic systems with less or without human footprints provide a robust baseline for comparison (Pesant et al., 2015). To better understand the impacts of anthropogenic activities and identify the most critical human activities for targetable management, it is essential to conduct holistic research on baseline-related alterations in coastal bacteriomes and resistomes and to reassess their contributions from different anthropogenic activities using advanced technologies.

2.3.2 Anthropogenic factors driving impacts

Environmental factors and nutrient concentrations have historically been recognized as principal determinants in shaping the intrinsic structure and variations of marine

bacteriomes and resistomes prior to the Anthropocene. With the increasing intensity of human activities in coastal regions (Nogales et al., 2011), anthropogenic impacts play an important role in driving the biological changes. Several studies have demonstrated that environmental and anthropogenic impacts jointly shape the biodiversity and ARG variations in human-impacted coastal waters (Dželalija et al., 2024; He et al., 2017; Ismail & Almutairi, 2022; Lu et al., 2020; Mohapatra et al., 2020; Zhang et al., 2009). In this context, there is a burgeoning interest in elucidating the anthropogenic mechanisms at play. Recent studies have linked alterations in microbial structure, resistance property, and bacterial ARG hosts to anthropogenic pollution from biological and chemical dimensions. Relevant anthropogenic factors include human fecal contamination (Makkaew et al., 2021), PAHs (Sauret et al., 2016; Wang et al., 2017), heavy metals (Coclet et al., 2020), and antibiotics (Chen et al., 2017; He et al., 2022; Ohore et al., 2022; Wang et al., 2022; Xu et al., 2019). In particular, antibiotics are believed to be the major selection agents for bacterial alterations and the rise of harmful elements. On the other hand, human fecal contamination can largely explain the presence of harmful components in the environment, even without clear signs of selection. This factor is often underexplored due to the underestimation of its role in shaping bacteriomes and resistomes (Karkman et al., 2019). Recently, researchers have emphasized the need to consider both on-site selection and human fecal contamination when assessing anthropogenic impacts (Karkman et al., 2019). Furthermore, the above-mentioned ARG transfer enhanced by anthropogenic stresses can facilitate the spread and proliferation of resistomes and ARB in coastal waters. However, there is still

insufficient research on this issue. Regarding these proposed concerns, it is necessary to unravel the contributions from these factors and environmental parameters to achieve a more precise and comprehensive elucidation of the underlying mechanisms.

2.4 Farming-induced biological contamination in mariculture systems

Mariculture farming, a widespread anthropogenic practice along coastlines, has expanded steadily over the past 60 years and become the sector with the most potential to supply enough seafood to satisfy the growing global demand for animal proteins (Costello et al., 2020). However, a lack of stringent sustainability criteria has led to the introduction of chemical and biological contamination, as antimicrobials and fish feeds are heavily applied to control disease outbreaks and promote finfish growth. Mariculture farms have deteriorated into 'genetic reactors' that favor the rise of human pathogens and antimicrobial resistance, threatening the safety of cultured seafood and mariculture sustainability (Watts et al., 2017). Understanding the emergence and dynamics of these harmful elements in mariculture systems, as well as their transfer to farmed seafood, holds significant implications for sustainable management and food security.

2.4.1 Human pathogens and resistomes in fish feeds and mariculture environments

To enhance yield, fishmeal is used extensively to promote animal growth in mariculture. However, this practice degrades water quality and introduces numerous harmful

elements into the mariculture systems. Several studies have demonstrated that fishmeal harbors a highly diverse and abundant array of ARGs and human potential pathogenic bacteria (HPPB) (Han et al., 2017, 2019). For example, researchers identified 132 ARGs and 25 HPPB species in five fishmeal samples collected from China, Peru, Russia, and Chile. Among these, genes encoding resistance to critically important human medicines (i.e., tetracyclines, multiple drugs, and beta-lactams) and the pathogenic species responsible for foodborne diseases (i.e., *Vibrio parahaemolyticus* and *E. coli)* dominated (Han et al., 2017, 2019). The use of fishmeal has already been linked to the propagation of ARGs in mariculture sediments (Han et al., 2017). Recent evidence also suggests that fishmeal may contribute to the prevalence of dominant resistance genes and opportunistic human pathogens in mariculture sites along China's coastlines (Han et al., 2017; He et al., 2023). Based on these investigations, fishmeal and mariculture environments share several dominant harmful elements, such as multi-drug and tetracycline resistance genes and opportunistic human pathogens, namely *Vibrio* spp. Obviously, fishmeal is an underestimated reservoir and source of ARGs and human pathogens, whose application likely fosters the development and emergence of these harmful elements in mariculture systems.

To control animal diseases under intensive farming operation, antimicrobial use has been commonplace in mariculture as a prescription for decades and has grown continuously every year (Schar et al., 2020). High antimicrobial concentrations commonly generate strong selection pressure for the emergence of human pathogens,

antimicrobial resistance, and drug-resistant pathogens in mariculture systems. Surveillance has reported that these bacterial contaminants in mariculture environments exhibit a higher relative abundance compared with control sites due to the selection (Gao et al., 2018; He et al., 2023; Muziasari et al., 2016). Mariculture systems have become one of the most severe 'hotspots' for the development of these harmful elements (Watts et al., 2017). Antimicrobial resistance, zoonotic pathogens, and resistant foodborne pathogens are detected in mariculture systems throughout the world, particularly in low-income and developing regions (Reverter et al., 2020; Schar et al., 2021).

Many researchers have examined the distributions and characteristics of these contaminants in fish feeds and mariculture environments (Buschmann et al., 2012; Dang et al., 2007; Gao et al., 2018; Muziasari et al., 2016; Ren et al., 2013; Reverter et al., 2020). However, there is insufficient understanding of the linkages between mariculture practices, environmental changes, and seafood contamination. For example, whether environmental alterations and feed applications induce the enrichment and structural change of these elements in farmed fish is still unclear. This knowledge gap poses challenges for sustainable management and the ability to alleviate contamination in the mariculture industry under the One Health framework. Previous studies have revealed that environmental properties and diet intakes are important drivers for the formation of microbiota and antibiotic resistance in wild marine fishes and shape their interspecies disparities (Collins et al., 2021; Korry & Belenky, 2023). In this context,

we hypothesize that environmental alterations and feed applications affect the occurrence, enrichment, structural shift, and interspecies similarity of these elements in cultured fishes, especially when movement and food sources are limited within a small-scale mariculture farm. If this hypothesis is supported, it indicates increased contaminant exposure for individuals involved in seafood processing and consumption, with potentially deleterious health consequences. The imperative to uncover this mystery becomes increasingly urgent.

2.4.2 Human pathogens and resistomes in cultured seafood

Fish-associated microbiota are highly dynamic, shaped by a complex interplay of the environmental microbiome, dietary composition, habitat, host taxonomy, immune response, trophic level, and feeding behavior (Chiarello et al., 2018; Huang et al., 2020; Kim et al., 2021; Ross et al., 2019). In contamination-free systems, diverse ambient and dietary bacterial components contribute beneficially to fish microbiota, supporting immune functions in maintaining microbial homeostasis and preventing pathogen invasions (Gomez et al., 2013; Ross et al., 2019). In wild marine fish populations, bacterial community structure frequently exhibits variation according to seasonal shifts, geographic distribution, and interspecies differences (Chiarello et al., 2018; Korry & Belenky, 2023; Ross et al., 2019). Marine-indigenous pathogens, as an important component of microbiota, may share the similar dynamics.

There has been limited research on antimicrobial resistomes in wild fish; few studies

have addressed their development and distribution. However, based on recent findings, multidrug and beta-lactam resistance genes found in the microbiota of deep-sea fish resemble those present in oceanic seawater (Collins et al., 2021; Cuadrat et al., 2020), suggesting that environmental resistomes may be critical in shaping resistance profiles in wild marine animals. Such resistance mechanisms appear to be conserved among marine bacteria, spanning both seawater and fish-associated microbiota. Further investigation has indicated that the resistome composition in wild fishes correlates with trophic levels, where piscivorous fishes at higher trophic levels carry a greater load of ARGs (Korry & Belenky, 2023). These initial findings suggest that environmental resistomes, coupled with trophic-related feeding behaviors, contribute to ARG occurrence and profile variability across different wild fish species.

However, in mariculture systems, farming practices often induce environmental contamination from chemical and biological perspectives, limit the fishes within small-scale farms, and supply artificial diets continuously. These activities can change the diversity of bacterial sources from natural environments and diets, disrupting microbial balance and suppressing immune functions in cultured fishes. Farmed fishes, particularly those exposed to contaminated environments and diets, have shown increased susceptibility to zoonotic pathogens compared to their wild counterparts (Austin, 1998; Sueiro et al., 2020). Various zoonotic and foodborne pathogens have been identified in farmed fishes, posing infection risks to both the fish themselves and human consumers (Barrett et al., 2017; Reverter et al., 2020; Schar et al., 2021).

Meanwhile, the excessive use of antimicrobials and other pollutants in mariculture has increased the selection pressure, promoting the emergence of antimicrobial resistance and resistant pathogens (Sáenz et al., 2019; Zeng et al., 2019). Thus, farmed seafood represents an ideal reservoir for human pathogens and antimicrobial resistance, acting as a vehicle for their transmission from mariculture environments to humans through seafood consumption (Heuer et al., 2009; Santos & Ramos, 2018). The currently available evidence suggests that these biological elements are closely linked to farming practices, yet there has been particularly limited research to understand how mariculture practices reshape the structure and distribution of these elements across fish species with varying feeding behaviors. Additionally, the impact of feed applications on the abundance and composition of resistomes and resistant pathogens in farmed fishes has been underexplored. Addressing these research gaps could offer valuable information for mariculture management strategies, aiming at good seafood quality.

2.4.3 Pathways of human pathogens and resistomes from the environment to cultured seafood

Source dissemination constitutes an important environmental pathway that influences the bacteriomes and resistomes in husbandry animals. Deciphering the source dynamics of harmful elements in farmed fish is critical for interpreting the mechanisms of seafood contamination. Feeds, seawater, and sediments serve as the reservoirs for human pathogens and antimicrobial resistance in mariculture systems and are considered as the sources for these elements in farmed organisms. Previous studies have qualitatively

linked the occurrence of these elements in animals with those present in environments and feeds using traditional culture-dependent approaches and qPCR techniques (Su et al., 2017). However, such information is insufficient for clear elucidation of mechanisms and contamination control, which highlights the importance and necessity of quantitative source tracking. Surprisingly, there have been very few quantitative source apportionment investigations on human pathogens and antimicrobial resistance in farmed fishes.

ARG transmission through HGT is an important pathway for the spread of antimicrobial resistance from source microbes to those in farmed animals, facilitating the reconstruction of ARG hosts and resistant pathogens in the organisms. Some surveys have discussed several MGEs and their associated mechanisms for ARG transmission in mariculture environments, such as the transposon and integron-associated ARG transfer in Baltic Sea sediments (Muziasari et al., 2016) and plasmid-mediated colistin resistance dissemination in seawater of long-distance coastal aquaculture in South China (An et al., 2023). Nevertheless, there has been limited investigation of ARG spread from environmental sources to cultured fishes. Resolving this issue is vital to mitigate resistance-related seafood safety concerns. On the other hand, the use of NGS and bioinformatics methods have uncovered a broader array of MGEs co-located with ARGs (Carr et al., 2021; Frost et al., 2005; Saak et al., 2020), highlighting the need for a more comprehensive understanding of transmission mechanisms that encompass diverse MGE types. The information will be beneficial to

achieve a more accurate view of ARG dissemination and its prevention in mariculture industries.

2.4.4 Community health risks associated with contaminated seafood

Along with intensive farming activities, the emergence of human pathogens and antimicrobial resistance constitutes a critical concern for seafood safety (Naylor et al., 2021). Indeed, these elements have been detected in cultured fish throughout the world (Reverter et al., 2020; Schar et al., 2021). Seafood contamination is responsible for a significant proportion of foodborne-disease outbreaks (Kumar et al., 2016). Among the confirmed foodborne pathogens, *S. aureus* is an important but underestimated pathogens (Elbashir et al., 2018). Such pathogenic species can acquire resistance and evolve into methicillin-resistant *S. aureus* (MRSA), which leads to high morbidity and mortality rates due to limited treatment options (Lee et al., 2018; Turner et al., 2019). From 1990 to 2021, MRSA showed the largest global increase in both AMR-associated and AMR-attributable deaths, becoming a leading cause of bacterial infections in both healthcare and community settings (Naghavi et al., 2024). In 2019, MRSA was the single pathogen – drug combination responsible for the highest number of AMR-attributable deaths — over 100,000 — surpassing other major resistant pathogens (Murray et al., 2022). Consequently, MRSA has emerged as a key representative of the antimicrobial resistance challenge under discussion. Many researchers have isolated MRSA from cultured seafood in different regions (Arfatahery et al., 2016; Fri et al., 2020; Murugadas et al., 2016; Vaiyapuri et al., 2019). Obviously, there is an increasing

prevalence of MRSA in mariculture environments and farmed seafood. Meanwhile, recent studies have demonstrated that community-based infections caused by MRSA have emerged as a global public health burden (Murray et al., 2022). Whether these phenomena are related remains unclear and requires further research, although such connections have been established in other food animals, including pigs, cattle and poultry (Aires-de-Sousa, 2017). Moreover, the increasing presence of MRSA in cultured seafood has sparked an interest in exploring their evolution in mariculture systems. Such information could help elucidate the contribution of marine-originated resistant pathogens to coastal disease outbreaks and mitigate the dissemination of these elements to coastal populations via seafood processing and consumption.

2.5 Summary and outlook

Bacteriomes and resistomes are intrinsic microbial components of pristine environments. However, shifts in their structure and variations have been observed in terrestrial and freshwater human-associated systems. The resultant emergence of human pathogens and antimicrobial resistance has become a global threat to environmental and human health. Coastal waters are vulnerable regions subject to various human activities, including sewage discharge of WWTPs, mariculture activities, contaminated river runoff, marine transportation, and recreational water usage. Anthropogenic disturbances on these microbial components, alongside an increasing prevalence of human pathogens and antimicrobial resistance, have been documented in these areas. Nevertheless, the extent and mechanisms of these impacts remain unclear.

Limited efforts have been made to clarify the structure and variations of these elements, particularly mobile ARGs, ARB, and resistant pathogens. To address these knowledge gaps, holistic metagenomic surveillance on the characteristics and the underlying drivers of these microbial components under human stress relative to the baselines is needed.

As human activities intensify in coastal regions, their impacts on coastal bacteriomes and resistomes have become increasingly pronounced. While antibiotic selection has traditionally been considered the primary driver of these impacts, recent studies suggest that fecal contamination plays a significant role, even in the absence of clear selective pressures. Anthropogenically enhanced transferability of ARGs further facilitates the spread and proliferation of resistomes and ARB in human-associated environments. These factors, although critical in shaping alterations in bacteriomes and resistomes, remain underexplored in mechanistic studies. To advance understanding, future investigations must integrate these factors alongside antibiotic selection to elucidate a more comprehensive driving mechanism. Additionally, the lack of clarity regarding key anthropogenic activities impedes targeted contamination management. Addressing this gap requires in-depth research into the concentrations of anthropogenic factors across different sites subject to varying levels of human impact and metagenomic analyses to uncover baseline shifts in bacteriomes and resistomes across these coastal zones. Such efforts are essential for informing effective mitigation strategies.

Mariculture is a widespread anthropogenic practice along coastlines to produce seafood. The unusual prevalence of human pathogens and resistomes has been documented in both environments and cultured animals. Although many studies have focused on the mechanisms of environmental changes in these biological elements, how these alterations induce their enrichment in cultured seafood remains unclear. Previous studies have demonstrated that environmental microbiomes and fish feeding behaviors profoundly influence the formation of microbial communities and antibiotic resistance in wild marine fishes and shape their interspecies differences. These findings lead to the hypothesis that mariculture-induced changes in environmental bacteria and resistomes affect their dissemination, enrichment, and interspecies similarity in cultured fishes, especially when their movement and food sources are limited to a small scale in a farm. On the other hand, mariculture contamination could suppress fish immune functions, facilitating the invasion and colonization of harmful elements in fish bodies, which would in turn reconstruct their structural compositions in cultured fishes. Furthermore, fishmeal applications can introduce human pathogens and antimicrobial resistomes into mariculture systems, which could contribute to their enrichment in cultured fishes. To untangle these issues, it is essential to quantify source contributions of these harmful elements in farmed fishes and clarify their links to the above factors. In addition, there is an increasing concern over the potential link between seafood contamination and global outbreaks of community-based diseases, yet this relationship has received limited research attention.

Chater 3 Methodology

Following the identification of research gaps in Chapter 2, we established a robust methodological framework encompassing sample collection and pretreatment, chemical analysis, DNA sequencing and annotation, as well as data analysis and visualization. This section provides a concise overview of the cities and sampling sites studied, and details the sampling strategies, experimental protocols, bioinformatics pipelines, and statistical methodologies utilized in this study. Together, these approaches ensure a comprehensive and systematic investigation of the research objectives.

3.1 Brief information about the cities studied

The selected cities are briefly described in terms of their population, economic conditions, climate, and seafood consumption volumes.

3.1.1 Population and blue economy

Hong Kong and Qingdao are two mega-cities with advanced economies and large populations. They are located in the southern and northern coastal regions of China, respectively.

Hong Kong, a densely populated coastal city in South China, hosts 7.49 million residents (Census and Statistics Department, 2023b) within its 1,114.57 km^2 territory (Lands Department, 2024). Its strategic position as a gateway between Mainland China

and the rest of the world has established Hong Kong as one of the most significant financial centers, commercial ports, and renowned tourist destinations globally. Hong Kong is the most open and international city in China and generated a gross domestic product (GDP) of HK$ 282.7009 billion in 2022 (Census and Statistics Department, 2023a). The marine economy contributed approximately 4.1% of the GPD and the maritime and port industry added an additional one fifth by facilitating the growth of trade and logistics (Transport and Logistics Bureau, 2023). Since 2017, Hong Kong has been a key member of the Guangdong–Hong Kong–Macao Greater Bay Area (GBA) established for the promotion of coordinated regional economic development under the key strategic plan in China's development blueprint. The GBA Outline Development Plan emphasizes enhancing Hong Kong's status as the world's leading international maritime center and advancing its marine industries to support high-quality economic development both locally and nationally. This strategy will promote the exploration of coastal waters but also face the challenges in ecological management to ensure sustainability in Hong Kong.

Qingdao, a coastal city located in the southeast of Shandong Province spans 11,293 km^2 and had a total resident population of 10.3421 million in 2022 (NBS Survey Office, 2023). As a key city on the Belt and Road Initiative's 'Maritime Silk Road', Qingdao holds the highest GDP among the cities in Shandong Province and has become one of the 'new first-tier cities' in China. According to the Qingdao Statistical Yearbook, the city's GDP has experienced rapid growth over the past decade, reaching 1,492.075

billion yuan in 2022 (NBS Survey Office, 2023). Notably, 33.6% of the GDP originates from marine industries, including mariculture, fishery, transportation, shipping, and tourism. The development of these sectors has been a significant driver of the local economy. With an increasing emphasis on the blue economy and the establishment of various development zones, such as the High-Tech Industrial Development Zone, the Blue Valley Marine Economic Development Zone, and the Qianwan Free Trade Port Zone, future exploration of inland and marine resources is expected to intensify along the coastal waters. The city aspires to become a global ocean center at the forefront of the world's urban system by 2035 (Qingdao, 2021). To promote sustainable development, the government underscores the importance of marine conservation and environmental protection in Qingdao's 14th Five-Year Plan for Marine Economic Development.

3.1.2 Climate

Due to its location in North China and along the northern coast of the Yellow Sea, Qingdao experiences a slightly continental climate with cold and dry winters and hot and humid summers. Cold and dry currents coming from the interior of China and Mongolia reduce the sea-mitigating effect and precipitation in winter, while warm and humid currents of tropical origin brought by the monsoon increase the number of rainy days and the amount of precipitation in summer. Facing the South China Sea and located at 22 degrees north latitude in South China, Hong Kong is governed by a subtropical climate with mild and relatively dry winters and hot and rainy summers.

The monsoonal seasonal changes are also well marked in Hong Kong, but less variable compared with Qingdao.

3.1.3 Seafood business and consumption

Seafood is a vital and popular food source for the coastal populations of Qingdao and Hong Kong. Economic growth in these cities has significantly bolstered the seafood industry and per capita consumption. According to the Agriculture, Fisheries and Conservation Department (AFCD) of the Hong Kong Government, Hong Kong is ranked among the global leaders in per capita seafood consumption, with residents consuming more than three times the global average annually (Agriculture Fisheries and Conservation Department, 2023). Although specific consumption data for Qingdao are unavailable, national trends indicate a steady rise in per capita seafood consumption across China (Crona et al., 2020). China now stands as the foremost global consumer of fish and seafood. In response to the increasing demand, seafood production in China continues to expand and the country maintains its status as the largest producer worldwide. Aquaculture is a critical driver for growth, with a 1.2% annual increase, while the wild-catch yield has declined (United States Department of Agriculture, 2023).

3.2 Description of the sampling sites

The surrounding environment of the sampling sites in coastal waters and the relevant human activities imply site-based anthropogenic impacts of different levels.

3.2.1 Sampling sites in the coastal waters of Qingdao

WWTP: The sampling site is located at the coastal water adjacent to the outfall of the Licun River WWTP, the largest WWTP in Qingdao. The WWTP processes wastewater from the Shibei, Licang, and Laoshan Districts, serving a total area of 147 km^2 and a population exceeding 1 million after its upgrade (Li, 2023). The current treatment capacity is 250,000 tons per day and will reach 300,000 tons per day after its expansion. The treatment technologies comprise high-speed air flotation, ozone oxidation, membrane bioreactor (MBR), and plate and frame filter press. The effluent meets the Class IV standard for surface water after treatment.

Beach: The sampling site is situated in the coastal water of Shilaoren Beach, one of the largest beaches along the Qingdao coastline and a key component of the Stone Man National Holiday Resort. According to local government sources, the beach covers an area of 0.2 km^2 and can accommodate up to 100,000 visitors simultaneously (Laoshan Government, 2008). This highly developed tourist destination attracts a high volume of travelers each year, with daily attendance exceeding 140,000 visitors during the peak season. The beach supports a diverse range of human activities, including bathing, sightseeing, sports, swimming, boating, and other entertainment, with activity levels peaking during the summer months.

Mariculture: The sampling site is situated in the Hongdao Mariculture Zone of Sifang District, a region predominantly characterized by extensive mariculture activities. This

zone is recognized as one of the most important mariculture areas in Qingdao. The mudflats in this region are occupied by mariculture farms, which support the cultivation of various marine species, such as fishes, clams, oysters, cucumbers, and lavers.

Estuary: The sampling site is located at the estuary of the Moshui and Baisha Rivers, the largest rivers in northern Qingdao. The rivers are influenced by pollution originating from the Chengyang and Jimo Districts, whose GDP collectively contributed over 19% of the total amount in 2022 (NBS Survey Office, 2023). A variety of livestock and poultry breeding farms, pharmaceutical factories, and hospitals are distributed along the rivers. The Moshui River is particularly notable for its elevated antibiotic concentrations (Zheng et al., 2020).

Port: The sampling site is located on the coastal water near Qingdao Ferry Station and Qingdao Port. Qingdao Ferry Station functions as an important transportation hub in the city, while Qingdao Port is ranked among the top ten ports in China. In 2022, Qingdao Port achieved a cargo throughput of 627 million tons and a container throughput of 26.82 million twenty-foot equivalent units (TEUs), generating an operating revenue of 19.263 billion yuan (Zhao, 2023).

Marine reserve: The sampling site is located on the coastal water near the rural region of Lingshan Bay. The region is covered by vegetation with a few human footprints. Only several buildings are scattered in this area.

3.2.2 Sampling sites in the coastal waters of Hong Kong

WWTP: The sampling site is located at the coastal water near the outfall of Stonecutters Island Sewage Treatment Works (SCISTW). This WWTP is designed as a chemically enhanced primary treatment plant to handle a flow of 2,450,000 m^3 per day, which is equivalent to the volume of about 1,000 Olympic-size swimming pools (Drainage Services Department, 2020). The infrastructure treats the sewage collected from several well-developed urban areas, including Kowloon and north-eastern, northern, and south-western Hong Kong Island. It is the largest sewage treatment works of its type in the world.

Beach: The sampling site is situated at the coastal water near the Repulse Bay Beach, one of the most popular beaches along the Hong Kong coastline. The beach attracts many tourists and residents throughout the year and is featured in a diverse range of recreational and sport activities. The daily number of visitors generally exceeds 700, with peak attendance reaching over 11,000 individuals on busy days (Environmental Protection Department, 2021, 2022).

Mariculture: The sampling sites are set at Tai Tau Chau and Lo Tik Wan, the eastern and southern fish culture zones. These areas are distanced from the Peal River and urban areas and are submitted to clean seawater from the South China Sea (Zhou et al., 2014). Intensive floating rafts distribute in the zones and seawater quality is highly impacted by mariculture activities. The farms are operated under the accredited fish farm scheme

issued by the AFCD. The production practices are representative of the Hong Kong mariculture industries.

Estuary: The sampling site is located at the estuary of Shing Mun River. This river flows through densely populated urban areas, including Tai Wai, Sha Tin, and Fok Tan and is influenced by pollutant inputs from these regions. It is one of the most important waterways and encompasses the largest catchment area in the territory.

Port: The sampling site is situated at the water of the Kwai Tsing Container Terminals, which is the largest port in Hong Kong and one of the top 10 ports worldwide. The port contains 24 berths and has an annual handling capacity exceeding 20 million TEUs. In both 2021 and 2022, its container throughput was more than 12.5 million TEUs (Hong Kong Maritime and Port Board, 2023).

Marine reserve: The sampling site is located on the coastal water of the Cape D'Aguilar Marine Reserve. The reserve lies on the southeastern tip of Hong Kong Island and is distant from the urban areas. To protect ecological biodiversity, recreational activities, such as visitation and water sports, are prohibited, with exception only for authorized scientific research. Consequently, the coastal water is less influenced by anthropogenic activities.

3.3 Sample collection strategies

To achieve the objectives of this project, we conducted seasonal sampling campaigns to collect seawater in human-impacted coastal sites and to sample cultured seafood and the putative sources of a typical mariculture system. The baseline datasets of healthy oceanic seawater samples were downloaded from the Tara Ocean database. This section provides detailed information on the sampling strategies, collection techniques, sampling periods, sample sizes, and downstream analysis approaches according to the different investigations.

3.3.1 Seawater collection in coastal waters subject to different anthropogenic impacts

Hong Kong and Qingdao are two representative megacities characterized by high urbanization and population density in southern and northern coastal China, respectively. Their coastal waters are heavily utilized for economic development, seafood production, and water recreation. To investigate the anthropogenic impacts on coastal bacteriomes and resistomes, we selected sampling sites subject to typical anthropogenic activities, including outfalls of WWTPs, mariculture farms, ports, beaches, estuaries, and marine reserves. These sites were classified based on varying levels of anthropogenic influence in relation to regional economy and ecological function.

Considering seasonal climatic variations in these regions, we conducted sampling

campaigns to collect surface seawater in August (summer, the wet season) and February (winter, the dry season) from 2021 to 2022 (Figure 3-1). Detailed information on the sampling sites, including the surrounding environments, industries, population density, economic contributions, and potential anthropogenic influences, is provided in Chapter 3.2. For genetic analysis, 4–5 replicates were collected from each site using 1-L sterile plastic bags (BKMAM Biotechnology Co., Ltd, Changsha, China) and a sterilized seawater sampler. For antibiotic measurement, samples were collected in 1-L clean grass bottles wrapped with aluminum foil to prevent photodegradation.

The sampling methods employed for genetic and antibiotic analysis have been well established in previous studies, ensuring sufficient biological material and antibiotic concentrations for downstream detection. In total, 132 samples from Hong Kong coastal waters and 118 samples from Qingdao coastal waters were collected. After collection, the samples were maintained at 4 °C in ice-packed containers and transported immediately to the laboratory. The total number of samples and succeeding analyses are summarized in Table 3-1. All sampling tools and materials were sterilized before use. During sampling, water parameters, including salinity, dissolved oxygen (DO), temperature, and pH were measured on site using the YSI Pro Plus multiparameter instrument (Xylem Inc., Washington, D.C., USA).

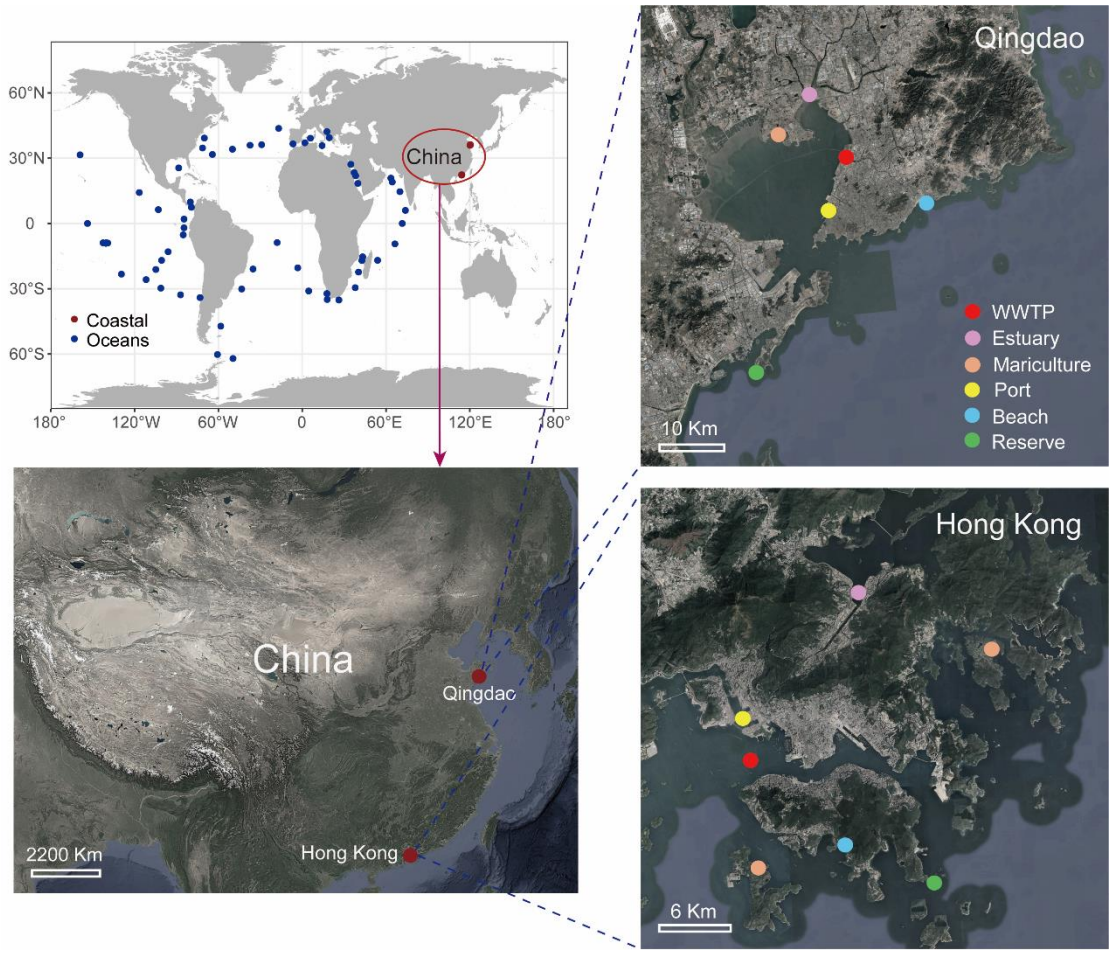

Figure 3-1 Map of the sampling locations in Hong Kong and Qingdao coastal waters subject to varying levels of anthropogenic impacts (e.g., sewage discharge, mariculture, port, beach, estuary, and marine reserve) versus those located in global oceans with few or without anthropogenic activities.

Table 3-1 Anthropogenic types and sample sizes in the spatial-temporal sampling campaigns as well as the downstream analyses applied.

City	Anthropogenic type	Sampling period	Season	Downstream analysis and sample size (wet and dry)
Qingdao	WWTP	August 2021	Wet	Genetic (5 and 5)
		February 2022	Dry	Chemical (3 and 5)
	Beach	August 2021	Wet	Genetic (5 and 5)
		February 2022	Dry	Chemical (5 and 5)
	Port	August 2021	Wet	Genetic (5 and 5)
		February 2022	Dry	Chemical (5 and 5)
	Mariculture	August 2021	Wet	Genetic (5 and 5)
		February 2022	Dry	Chemical (5 and 5)
	Estuary	August 2021	Wet	Genetic (4 and 5)
		February 2022	Dry	Chemical (4 and 5)
	Reserve	August 2021	Wet	Genetic (5 and 5)
		February 2022	Dry	Chemical (5 and 5)
Hong Kong	WWTP	August 2021	Wet	Genetic (4 and 5)
		February 2022	Dry	Chemical (4 and 4)
	Beach	August 2021	Wet	Genetic (5 and 5)
		February 2022	Dry	Chemical (5 and 5)
	Port	August 2021	Wet	Genetic (5 and 5)
		February 2022	Dry	Chemical (5 and 5)
	Mariculture	August 2021	Wet	Genetic (9 and 9)
		February 2022	Dry	Chemical (9 and 9)
	Estuary	August 2021	Wet	Genetic (5 and 5)
		February 2022	Dry	Chemical (5 and 5)
	Reserve	August 2021	Wet	Genetic (4 and 5)
		February 2022	Dry	Chemical (4 and 5)

WWTP, wastewater treatment plant.

3.3.2 Selection of oceanic seawater samples as baselines for the comparative study

A reliable baseline is essential for environmental research and health risk assessment. In many human-associated systems, including coastal waters, such baselines are often obscured by the continuous input of anthropogenic elements. However, anthropogenic

footprints are strongly reduced and diluted in oceanic systems due to their distance from urbanized mainland and human activities. As a result, oceanic seawater, with few or without anthropogenic impacts, likely retains pristine bacteriomes and resistomes. On the other hand, considering the vast compositional differences between microbial communities in terrestrial, freshwater, atmospheric, human gut, and marine ecosystems (Shin et al., 2015), oceanic seawater provides the most reliable reference. Consequently, it can serve as a global baseline and represents a clean and healthy system for comparison in our investigation.

Recent Tara Ocean expeditions (2009 – 2013) systematically sampled most of the biogeographic and biogeochemical provinces of the global ocean using standardized protocols and logistics for collection, distribution and storage (Pesant et al., 2015). Employing advanced sequencing technologies and cutting-edge bioinformatics pipelines, the resulting metagenomes exhibit exceptional data quality, with high sequencing depth and minimal contamination (Sunagawa et al., 2020). The first series of publications has demonstrated the potential of Tara Oceans data to study the ecology of plankton and the structural and functional diversity of viruses, prokaryotes and eukaryotes in the global ocean, proposing this dataset as a baseline for assessing ecosystem changes and the future habitability of our planet in the Anthropocene epoch (Sunagawa et al., 2020).

Building on this foundation, and to minimize the influence of natural variability or

undetected anthropogenic impacts on an ecosystem-wide overview of the global ocean microbiome, our study sourced baseline data from surface seawater samples collected globally across seven oceanic systems: the Mediterranean Sea (MS), the Red Sea (RS), the Indian Ocean (IO), the North Atlantic Ocean (NAO), the South Atlantic Ocean (SAO), the South Ocean (SO), the North Pacific Ocean (NPO), and the South Pacific Ocean (SPO). A total of 61 surface seawater samples, containing particles with a fraction size from 0.22 to 1.6 or 3 μm, satisfied the criteria for metagenomic comparison, as they captured comprehensive microbial community profiles, which has been demonstrated by previous investigations into the global ocean microbiome using NGS technologies (Sunagawa et al., 2015).

The 61 metagenomes (raw short sequence reads) were retrieved from the European Nucleotide Archive (ENA; they were freely available) for further bioinformatics analysis and comparative studies. A summary of sample information, including sampling dates, ocean regions, and accession numbers, is presented in Table 3-2. In addition, the sample locations are presented on the world map in Figure 3-1.

Table 3-2 Sample information of oceanic seawater samples for comparative studies.

Station	Fraction size	Accession number	Date	Latitude (°N)	Longitude (°E)	Depth (m)	Ocean
TARA_004	0.22-1.6	ERR598955	2009-09-15	36.5533	-6.5669	5	NAO
TARA_007	0.22-1.6	ERR315857	2009-09-23	37.051	1.9378	5	MS
TARA_009	0.22-1.6	ERR594288	2009-09-28	39.1633	5.916	5	MS
TARA_018	0.22-1.6	ERR598993	2009-11-02	35.759	14.2574	5	MS
TARA_023	0.22-1.6	ERR315858	2009-11-18	42.2038	17.715	5	MS
TARA_025	0.22-1.6	ERR599043	2009-11-23	39.3888	19.3905	5	MS
TARA_031	0.22-1.6	ERR599106	2010-01-09	27.16	34.835	5	RS
TARA_032	0.22-1.6	ERR599116	2010-01-11	23.36	37.2183	5	RS
TARA_033	0.22-1.6	ERR599049	2010-01-13	21.9467	38.2517	5	RS
TARA_034	0.22-1.6	ERR598991	2010-01-20	18.3967	39.875	5	RS
TARA_036	0.22-1.6	ERR599143	2010-03-12	20.8183	63.5047	5	IO
TARA_038	0.22-1.6	ERR599102	2010-03-15	19.0393	64.4913	5	IO
TARA_041	0.22-1.6	ERR599074	2010-03-30	14.6059	69.9776	5	IO
TARA_042	0.22-1.6	ERR599075	2010-04-04	6.0001	73.8955	5	IO
TARA_045	0.22-1.6	ERR599054	2010-04-13	0.0033	71.6428	5	IO
TARA_048	0.22-1.6	ERR599138	2010-04-19	-9.3921	66.4228	5	IO
TARA_052	0.22-1.6	ERR599139	2010-05-17	-16.957	53.9801	5	IO
TARA_056	0.22-3	ERR599057	2010-06-26	-15.3424	43.2965	5	IO
TARA_057	0.22-3	ERR599058	2010-06-27	-17.0248	42.7401	5	IO
TARA_062	0.22-3	ERR599012	2010-07-03	-22.3368	40.3412	5	IO
TARA_064	0.22-3	ERR599150	2010-07-07	-29.5019	37.9889	5	IO
TARA_065	0.22-3	ERR599146	2010-07-12	-35.1728	26.2868	5	IO
TARA_066	0.22-3	ERR599173	2010-07-15	-34.9449	17.9189	5	SAO
TARA_067	0.22-3	ERR599144	2010-09-07	-32.2401	17.7103	5	SAO
TARA_068	0.22-3	ERR599174	2010-09-14	-31.0266	4.665	5	SAO
TARA_070	0.22-3	ERR599165	2010-09-21	-20.4091	-3.1759	5	SAO
TARA_072	0.22-3	ERR598984	2010-10-05	-8.7789	-17.9099	5	SAO
TARA_076	0.22-3	ERR599126	2010-10-16	-20.9354	-35.1803	5	SAO
TARA_078	0.22-3	ERR599006	2010-11-04	-30.1367	-43.2899	5	SAO
TARA_082	0.22-3	ERR599009	2010-12-06	-47.1863	-58.2902	5	SAO
TARA_084	0.22-3	ERR599059	2011-01-03	-60.2287	-60.6476	5	SO
TARA_085	0.22-3	ERR599090	2011-01-06	-62.0385	-49.529	5	SO
TARA_093	0.22-3	ERR599064	2011-03-12	-34.0614	-73.1066	5	SPO
TARA_094	0.22-3	ERR599050	2011-03-18	-32.7971	-87.0693	5	SPO
TARA_096	0.22-3	ERR598967	2011-03-24	-29.7238	-101.1604	5	SPO
TARA_098	0.22-3	ERR599093	2011-04-03	-25.8051	-111.7202	5	SPO
TARA_099	0.22-3	ERR599024	2011-04-09	-21.146	-104.787	5	SPO
TARA_100	0.22-3	ERR599163	2011-04-15	-13.0023	-95.9759	5	SPO
TARA_102	0.22-3	ERR598978	2011-04-21	-5.2529	-85.1545	5	SPO
TARA_109	0.22-3	ERR599118	2011-05-12	1.9928	-84.5766	5	NPO

TARA_110	0.22-3	ERR599039	2011-05-21	-2.0133	-84.589	5	SPO
TARA_111	0.22-3	ERR599077	2011-05-31	-16.9601	-100.6335	5	SPO
TARA_112	0.22-3	ERR598954	2011-06-14	-23.2811	-129.3947	5	SPO
TARA_122	0.22-3	ERR598992	2011-07-26	-8.9971	-139.1963	5	SPO
TARA_123	0.22-3	ERR599160	2011-07-31	-8.9068	-140.283	5	SPO
TARA_124	0.22-3	ERR588857	2011-08-04	-9.1504	-140.5216	5	SPO
TARA_125	0.22-3	ERR599119	2011-08-08	-8.9111	-142.5571	5	SPO
TARA_128	0.22-3	ERR599038	2011-09-04	0.0003	-153.6759	5	SPO
TARA_132	0.22-3	ERR599142	2011-10-04	31.5213	-158.9958	5	NPO
TARA_137	0.22-3	ERR598989	2011-12-02	14.2035	-116.6261	5	NPO
TARA_138	0.22-3	ERR599030	2011-12-10	6.3332	-102.9432	5	NPO
TARA_140	0.22-3	ERR599162	2011-12-21	7.4122	-79.3017	5	NPO
TARA_141	0.22-3	ERR599029	2011-12-30	9.8481	-80.0454	5	NAO
TARA_142	0.22-3	ERR599136	2012-01-09	25.5264	-88.394	5	NAO
TARA_145	0.22-3	ERR598983	2012-02-02	39.2305	-70.0377	5	NAO
TARA_146	0.22-3	ERR598968	2012-02-15	34.6712	-71.3093	5	NAO
TARA_148	0.22-3	ERR599123	2012-02-24	31.6948	-64.2489	5	NAO
TARA_149	0.22-3	ERR598963	2012-03-01	34.1132	-49.9181	5	NAO
TARA_150	0.22-3	ERR599170	2012-03-05	35.9346	-37.3032	5	NAO
TARA_151	0.22-3	ERR598976	2012-03-09	36.1715	-29.023	5	NAO
TARA_152	0.22-3	ERR599078	2012-03-19	43.6792	-16.8344	5	NAO

IO, Indian Ocean; MS, Mediterranean Sea; NAO, North Atlantic Ocean; NPO, North Pacific Ocean; RS, Red Sea; SAO, South Atlantic Ocean; SO, South Ocean; SPO, South Pacific Ocean.

3.3.3 Collection of seafood and the corresponding sources from a representative farm in Hong Kong mariculture zones

To investigate the anthropogenic emergence and source contributions of human pathogens and resistomes in cultured seafood and their implication for seafood safety, we selected a representative mariculture farm in a fish culture zone of Hong Kong coastal waters for a detailed study. The farm, situated on the eastern coast near Tai Tau Chau Island (Figure 3-2), is geographically isolated from urban centers and controlled by clean seawater from the South China Sea (Morton & Wu, 1975; Zhou et al., 2014). Within the farm, contamination is predominantly attributed to on-site activities,

including antibiotic bath treatments, feed inputs, and the direct discharge of human waste by farmers. This geographic isolation effectively minimized the likelihood of other external contamination sources. To reduce environmental pollution and to encourage sustainable practices, farming operations are regulated under the Marine Fish Culture Ordinance and the Accredited Fish Farm Scheme. Cultured fishes in the farm are mainly fed with dry pellets due to their benefits in enhancing environmental quality and fish health. Trash fish is used as feed only when available, typically during summer. The farm rears various fish species commonly sold in markets and consumed by residents, including *Siganus canaliculatus* (Park, 1797) (SC, an herbivore), *Trachinotus blochii* (Lacepède, 1801) (TB, an omnivore), *Epinephelus coioides* (Hamilton, 1822) (EC, a carnivore), and *Epinephelus fuscoguttatus* × *Epinephelus lanceolatus* (EFL, a carnivore). These species are highly abundant in Hong Kong fish culture zones.

Feeding behaviors and seasonal environmental variations were carefully integrated into the sampling design to examine their potential impacts on the microbiomes and resistomes in culture fishes. The sampling campaign was executed in August 2021 (summer, the wet season) and February 2022 (winter, the dry season) to collect common fish species with different feeding behaviors, along with the corresponding environmental and feed samples as putative sources. Various sample types, including various fish species (of a similar size, see Table 3-3), surface and mesopelagic seawater, sediment, and distinct feed types, were collected in duplicate concurrently during each

season. Prior to sampling, fish were starved of feeds for 48 h to eliminate the effects of food residue on gut microbiome and resistome analyses. To prevent contamination, fishing nets were rinsed with on-site seawater. Following capture, each fish was immediately sealed in a sterilized plastic bag. Seawater samples from each layer were collected from various farm locations using a sterilized sampler and consolidated into a composite sample, from which one 1-L replicate was taken. Surface sediments were also collected from different locations and combined into one composite sample, from which one 50-mL replicate was taken. The same procedure was employed to collect another replicate of seawater samples of each layer and sediment samples. Due to the seasonal variations in feed materials, trash fish and feed pellets were collected during summer, while only feed pellets were sampled in winter with distinct types for groupers and other fish species. All samples were preserved in containers with freezer packs at 4 °C and delivered to the laboratory immediately after sampling. All tools and materials were sterilized before use. The sampling site and sample types are illustrated in Figure 3-2. Detailed information on the collected samples, including the sampling periods, sample sizes, and the downstream analysis, is summarized in Table 3-4.

Table 3-3 Information on adult fish collected from the representative mariculture farm.

Species	Feeding behavior	Season	No.	Weight (g)	Length (cm)
Siganus canaliculatus (Park,1797) (SC)	Herbivore	Summer	1	264	23
		Summer	2	293	25.5
		Winter	1	302	24.9
		Winter	2	228	24.2
Trachinotus blochii (Lacepède,1801) (TB)	Omnivore	Summer	1	380	28.5
		Summer	2	318	28
		Winter	1	170	20.7
		Winter	2	236	22.2
Epinephelus coioides (Hamilton,1822) (EC)	Carnivore	Summer	1	2117	49
		Summer	2	1671	45
		Winter	1	1898	48.3
		Winter	2	2371	52.4
Epinephelus fuscoguttatus × *Epinephelus lanceolatus (EFL)*	Carnivore	Summer	1	1540	44
		Summer	2	1491	42
		Winter	1	1984	44.5
		Winter	2	1886	44.5

Figure 3-2 Sampling location and sample types collected from the representative mariculture farm under the Accredited Fish Farm Scheme in Sai Kung, Hong Kong.

Table 3-4 Information on the samples collected from the representative mariculture farm.

Sample type	Sampling period	Season	Sample size	Downstream analysis
Fish skin	August 2021	Wet	8	Genetic analysis
	February 2022	Dry	8	Genetic analysis
Fish gut	August 2021	Wet	8	Genetic analysis
	February 2022	Dry	8	Genetic analysis
Surface seawater	August 2021	Wet	2	Genetic analysis
	February 2022	Dry	2	Genetic analysis
Mesopelagic seawater	August 2021	Wet	2	Genetic analysis
	February 2022	Dry	2	Genetic analysis
Sediment	August 2021	Wet	2	Genetic analysis
	February 2022	Dry	2	Genetic analysis
Dry pellet	August 2021	Wet	2	Genetic analysis
	February 2022	Dry	4	Genetic analysis
Trash fish	August 2021	Wet	2	Genetic analysis

3.4 Sample pretreatment

After sampling, the samples were pretreated according to specific protocols for the downstream analyses to measure different chemical and biological components. The specific pretreatment methods are detailed below.

3.4.1 Seawater collected from coastal waters

After arrival in the laboratory, seawater samples were stored at 4 °C in a cold room and processed within 24 hours. For metagenomic sequencing and genetic analysis, each sample was filtered through a 0.2 um sterile membrane filter (47 mm, Pall Corporation, New York, USA) to collect microbial fractions. The filtrates were then used for chemical analyses to detect dissolved organic carbon (DOC) and nutrient indices. Before filtration, all filtration equipment and tools were sterilized using autoclaving or

ethanol. The filters were subsequently preserved at –80 °C until DNA extraction. During DNA extraction, microbial fractions were collected from each filter using a sterilized swab. Another seawater batch for antibiotic measurement was filtered through 0.7-μm glass fiber membranes (47 mm, GE Healthcare UK Limited, Buckinghamshire, UK) and stored in the dark at 4 °C for further chemical analysis.

3.4.2 Cultured fish and the putative sources sampled from the representative mariculture farm

Upon arrival in the laboratory, the fish skin microbiome was sampled by swabbing the entire side of the body (from the back of the operculum to the caudal peduncle) using sterile swabs, following the methodology established for collecting skin microbiome of coral reef fishes (Chiarello et al., 2018). To obtain the gut microbiome, the intestinal tract was separated from the surrounding tissues using dissection tools sterilized with 75% ethanol and rinsed with MiliQ water. The gut contents were carefully squeezed and transferred into a sterile plastic tube. Seawater samples (1 L each) were filtered through 0.2-μm sterile membrane filters (47 mm, Pall Corporation) within 24 h, and the filters were stored at –80 °C. Microbial fractions on the filters were collected with sterilized swabs until no visible residue remained during DNA extraction. The same protocol employed for cultured fish skin microbiome was applied to trash fish skin microbiome. Dry pellets were gently ground into powders using mortars. Sediments were used directly for DNA extraction without pretreatment. All pretreated samples, as well as raw sediments, were stored at –80 °C until DNA extraction. All tools were

sterilized before sample preparation.

3.5 Chemical-based analysis

Key indices affecting the structural composition of coastal bacteriomes and resistomes, including DOC, nutrients, and antibiotics, were measured in the laboratory. Chemical analyses were conducted on seawater samples collected from human-impacted coastal waters of Qingdao and Hong Kong from August 2021 to February 2022. A total of 122 samples were included, which are detailed in Table 3-1.

DOC was measured using a total organic carbon analyzer (SHIMADZU, Japan) following the non-purgeable organic carbon (NPOC) protocol. Nutrient indices, including nitrate (NO_3^-) and reactive phosphorus (PO_4^{3-}), were quantified with a continuous flow auto-analyzer (Scalars San++, Skylar Analytical B.V., Breda, the Netherlands). Antibiotic concentrations were determined using a solid-phase extraction coupled with electrospray ionization liquid chromatography-tandem mass spectrometry (SPE-ESI-LC-MS/MS) method as described previously (Wu et al., 2022). A total of 20 antibiotics from seven classes (beta-lactams, macrolides, sulfonamides, tetracyclines, fluoroquinolones, lincosamides, and miscellaneous) were selected as the targe analytes, including amoxicillin, ampicillin, cefalexin, cefotaxime, erythromycin-H_2O, clarithromycin, roxithromycin, azithromycin, sulfamethazine, sulfamethoxazole, sulfadiazine, tetracycline, chlortetracycline, doxycycline, oxytetracycline, ofloxacin, ciprofloxacin, enrofloxacin, lincomycin, and trimethoprim (Table 3-5).

For extraction and detection, 1 L of the filtrate was treated with 0.2 g of ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) for metal chelation, and the pH was adjusted to 3~4 using formic acid. Next, 20 ng of $^{13}C_3$-caffeine was added to the sample as a surrogate for monitoring recovery. Antibiotics were extracted using an Oasis HLB cartridge (6 mL, 500 mg, Waters, Milford, Massachusetts, USA), which was preconditioned with 5 mL of methanol, 5 mL of Milli-Q water, and 5 mL of 0.02% formic acid in Milli-Q water. The sample was loaded at a flow rate of 1~2 drops/s. Then, the cartridge was washed with 5 mL of acidified Milli-Q water and dried under vacuum at ~5 mmHg for 30 min. Target analytes were eluted with 5 mL methanol into a 15-mL polypropylene centrifuge tube, evaporated to near dryness under a gentle nitrogen stream at 40 ℃, and reconstituted in 0.5 mL of 1:1 methanol/Milli-Q water (v/v) with 40 ng of the internal standard mixture for instrumental analysis. Detection was performed using a 1290 Infinity liquid chromatograph (LC; Agilent, Palo Alto, CA, USA) coupled with a QTRAP 5500 tandem mass spectrometer (SCIEX, Woodlands, Singapore) in multiple reaction monitoring (MRM) mode, with a Zorbax Eclipse Plus C18 column (2.1 mm i.d. × 50 mm L., 1.8 μm; Agilent). The mobile phase consisted of Milli-Q water (0.02% formic acid, v/v) (A) and methanol (0.02% formic acid, v/v) (B). Details of the LC gradient program, method recoveries, instrumental detection limits (IDLs), and method detection limits (MDLs) have been published (Wu et al., 2022). Quality assurance and control included the procedural blank and surrogate recovery for each batch of the experiment. The surrogate recoveries ranged from 70% to 144%, 90% of which were below 125% (Table 3-6). Corresponding corrections were made for

target analyte concentrations when the analytes were detected in the procedure blank. All eight-point standard curve regression coefficients were greater than 0.993, except ciprofloxacin, which had a coefficient of 0.967. In total, 14 antibiotics were frequently detected in seawater samples, namely cefalexin, cefotaxime, erythromycin-H_2O, clarithromycin, roxithromycin, azithromycin, sulfamethoxazole, sulfadiazine, chlortetracycline, doxycycline, ofloxacin, ciprofloxacin, lincomycin, and trimethoprim.

Table 3-5 Twenty antibiotics analyzed in coastal seawater samples.

Antibiotics	CAS number	Classes	Internal standard	Source
Amoxicillin	26787-78-0	Beta-lactam	$^{13}C_6$-Sulfamethazine	TRC*
Ampicillin	69-53-4	Beta-lactam	$^{13}C_6$-Sulfamethazine	TRC*
Cefalexin	15686-71-2	Beta-lactam	$^{13}C_6$-Sulfamethazine	TRC*
Cefotaxime	63527-52-6	Beta-lactam	$^{13}C_6$-Sulfamethazine	TRC*
Erythromycin-H_2O	23893-13-2	Macrolide	Roxithromycin-d_7	TRC*
Clarithromycin	81103-11-9	Macrolide	Roxithromycin-d_7	TRC*
Roxithromycin	80214-83-1	Macrolide	Roxithromycin-d_7	TRC*
Azithromycin	83905-01-5	Macrolide	Roxithromycin-d_7	TRC*
Sulfamethazine	57-68-1	Sulfonamide	$^{13}C_6$-Sulfamethazine	TRC*
Sulfamethoxazole	723-46-6	Sulfonamide	$^{13}C_6$-Sulfamethazine	TRC*
Sulfadiazine	68-35-9	Sulfonamide	$^{13}C_6$-Sulfamethazine	TRC*
Tetracycline	60-54-8	Tetracycline	Doxycycline-d_3	SA**
Chlortetracycline	57-62-5	Tetracycline	Doxycycline-d_3	TRC*
Doxycycline	564-25-0	Tetracycline	Doxycycline-d_3	TRC*
Oxytetracycline	79-57-2	Tetracycline	Doxycycline-d_3	SA**
Ofloxacin	82419-36-1	Fluoroquinolone	Ofloxacin-d_8	TRC*
Ciprofloxacin	85721-33-1	Fluoroquinolone	Ofloxacin-d_8	SA**
Enrofloxacin	93106-60-6	Fluoroquinolone	Ofloxacin-d_8	TRC*
Lincomycin	154-21-2	Lincosamide	$^{13}C_6$-Sulfamethazine	TRC*
Trimethoprim	738-70-5	Miscellaneou	$^{13}C_6$-Sulfamethazine	TRC*
$^{13}C_3$-Caffeine	78072-66-9	Surrogate	$^{13}C_6$-Sulfamethazine	TRC*

* Toronto Research Chemicals (Toronto, Ontario, Canada); ** Sigma Aldrich (St. Louis, MO, USA).

Table 3-6 The average surrogate recoveries of coastal seawater samples collected from different sampling sites.

City	Anthropogenic type	Sampling coastal site	Season	Recovery (Caffeine_13C3)
Qingdao	WWTP	Licun River WWTP	Wet	102%±4%
			Dry	108%±7%
	Beach	Shilaoren Beach	Wet	116%±11%
			Dry	90%±6%
	Port	Qingdao Ferry Station and Qingdao Port	Wet	108%±7%
			Dry	102%±8%
	Mariculture	Hongdao mariculture zone	Wet	119%±9%
			Dry	103%±7%
	Estuary	Moshui River and Baisha River	Wet	121%±10%
			Dry	113%±9%
	Reserve	Lingshan Bay	Wet	97%±6%
			Dry	100%±4%
Hong Kong	WWTP	Stonecutters Island Sewage Treatment Works	Wet	92%±8%
			Dry	104%±20%
	Beach	Repulse Bay Beach	Wet	118%±24%
			Dry	88%±9%
	Port	Kwai Tsing Container Terminals	Wet	100%±6%
			Dry	96%±10%
	Mariculture	Tai Tau Chau and Lo Tik Wan	Wet	95%±15%
			Dry	98%±9%
	Estuary	Shing Mun River	Wet	114%+13%
			Dry	125%±7%
	Reserve	Cape D'Aguilar Marine Reserve	Wet	112%±28%
			Dry	100%±3%

WWTP, wastewater treatment plant.

3.6 Molecular biological analysis

To elucidate the genetic landscape, we conducted DNA extraction, metagenomic sequencing, and comprehensive bioinformatics analysis. These efforts aimed to characterize bacterial communities, human pathogens, resistomes, mobilomes, ARG hosts, and potential resistant human pathogens at different genetic levels across samples.

Additionally, we employed special assays, including phylogenetic analysis and source appointment, to investigate the health risks associated with bacterial elements.

3.6.1 DNA extraction

Total genomic DNA of coastal seawater samples was extracted using QIAamp DNA Mini Kits (QIAGEN, Hilden, Germany) according to the manufacturer's protocol for swabs. Considering the diversity of sample types in the mariculture study, samples were divided into two groups based on the pretreatment procedures and physical properties. To ensure comparability in bacteriome and resistome analyses, extraction kits with similar protocols were selected. Swab samples, including fish skins, seawater, and trash fishes, were processed using QIAamp DNA Mini Kit (QIAGEN) following the manufacturer's instruction for swabs, while DNA from gut contents, sediments, and feed pellets was extracted employing QIAamp Fast DNA Stool Mini Kit (QIAGEN) according to the manufacturer's protocol for stools. After extraction, DNA concentrations were quantified with a Qubit 3.0 Fluorometer with the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA).

3.6.2 Metagenomic sequencing

More than 100 ng DNA of each sample from the coastal sites and mariculture farm was sent to the Beijing Genomics Institute for low-input library construction and shotgun metagenomic sequencing on the MGISEQ-2000 platform. The sequencing process was conducted as follows. Genomic DNA was randomly fragmented using a Covaris system

after quality testing by running an aliquot on a 1% agarose gel. Fragments of the desired size were subject to end-repair, 3′ adenylation, adapter ligation, and PCR amplification. The double-stranded PCR products were purified with magnetic beads, denatured by heating, and circularized with a splint oligo sequence. The resulting single-strand circle DNA (ssCir DNA) was further formatted to form the final library, which underwent quality control before sequencing on the MGISEQ-2000 platform. Paired-end reads were obtained via combinatorial Probe-Anchor Synthesis (cPAS).

For reliable bioinformatics analysis, raw sequencing data were quality filtered using fastp (v0.23.1) with default settings to remove low-quality sequences and adaptors (Chen et al., 2018). After filtering, approximately 10 gigabases (Gb) of clean data with paired-end reads (2 × 150 base pairs [bp]) were obtained for each sample collected from the coastal waters of Qingdao and Hong Kong during the wet season. For subsequent samples from the dry season and the mariculture farm, a library construction strategy yielding paired-end reads of 2 × 100 bp was adopted to generate approximately 20 Gb of clean data per sample. This approach aimed to align the data volume with what was obtained from the oceanic seawater samples for comparative analysis. Overall, around 1,890 GB of clean data were generated from coastal seawater samples, and 1,040 GB were generated from mariculture samples after quality control. The 61 oceanic seawater samples were also quality filtered by performing the fastp pipeline before bioinformatics analysis.

In animal microbiome research, host decontamination is commonly recommended. However, significant bacterial richness loss was observed when decontaminating fish data using a common pipeline involving Bowtie2 (v2.4.5) (Langmead & Salzberg, 2012), SAMtools (v1.6) (Danecek et al., 2021), and BEDtools (v2.26.0) (Quinlan & Hall, 2010), aligning against fish reference genomes retrieved from the National Center for Biotechnology Information (NCBI) GenBank database for species of *Trachinotus ovatus* (GCA_900231065.1) (Guo et al., 2020), EC (GCA_900536245.1) (Xiao et al., 2020), and *Epinephelus lanceolatus* (GCA_005281545.1) (Wang et al., 2019). To avoid underestimation and incorrect assessment, clean data rather than decontaminated data were preferred for downstream bioinformatics analysis.

3.6.3 Bioinformatics analysis

Prevalent bioinformatic pipelines, routinely used for the annotations in previous studies, were employed to identify target elements at different genetic levels. The detailed annotation procedures are outlined below.

3.6.3.1 Read-based bacterial taxonomy and resistomes

Read-based bacterial taxonomy was annotated using the Kraken 2 (v2.0.8-beta) (Wood et al., 2019) and Bracken 2 (v2.5.0) (Lu et al., 2017) pipelines using the bacterial database downloaded from NCBI (2021/12/03) with default settings. This database integrates high-quality reference genomes from the Reference Sequence (RefSeq) database and other curated sources. Its broad taxonomic coverage ensures the reliable

detection of both abundant and rare taxa, making it suitable for complex environmental samples, including coastal and oceanic microbiomes. It has become the most used database in microbial research. After annotations, the identified bacterial species in each sample were supported by no less than nine reads, with the exception of *Trichormus variabilis*, which was represented by only two reads in a coastal sample (HKWinME2) and in an oceanic sample (ERR599174). Rarefaction curves of bacterial richness were analyzed with the 'vegan' package in R (Dixon, 2003) to evaluate the influence of sequencing depth on genetic information capture. The curves show that bacterial richness reached a maximum and remained stable as the number of reads increased, suggesting the annotated bacterial information in each sample was adequately captured at the current sequencing depth (Figures 3-3 and 3-4). Bacterial species were further classified as emerging or opportunistic human pathogens based on an updated pathogen list (Li, Ju, et al., 2015), which includes 538 pathogenic specie—significantly more than previous databases. This comprehensive list enables broad-spectrum monitoring of emerging or opportunistic human pathogens in various environmental samples. If a bacterial taxon matched a species on the list, then it was identified as an emerging or opportunistic human pathogen. All identified pathogenic species were supported by no less than nine reads. Species richness was calculated using the 'vegan' package in R, following rarefaction of each sample to 1,501,797 reads.

The annotation of ARGs in coastal and oceanic seawater samples was performed using ARG-OAP (v2.3), aligning clean reads against the Structured ARG database (SARG

v2.2). This database comprises 12085 carefully curated sequences from multiple public resources, including the Comprehensive Antibiotic Resistance Database (CARD), the Antibiotic Resistance Genes Database (ARDB) and the NCBI Non-Redundant Protein Sequences Database (NCBI-NR). It employs a hierarchical structure to facilitate the classification of sequences identified through similarity search and has demonstrated improved coverage of ARG detection in metagenomes from diverse environmental samples. Compared with other databases, such as CARD, DeepARG, GROOT, it exhibits superior performance in ARG annotation and provides more reliable results (Montassier et al., 2021). Currently, it has been widely used and highly cited in studies involving ARG annotation. The specified parameters used were identity ≥ 60% and e-value ≤ 10^{-7}, which can achieve an accuracy rate exceeding 80% (Yin et al., 2018). For the mariculture study, the updated version (v3.2.3) and the current database (SARG v3.2.1-S) were applied, with the same parameter settings plus a query coverage of no less than 75% (Yin et al., 2022). To enhance biological relevance and comparability, ARG abundance was expressed as ARG copies per cell in the mariculture study, compared to ARG copies per 16S rRNA gene in coastal investigations (Yin et al., 2023). ARG richness was reported as the total number of resistome subtypes across all studies. To evaluate source dissemination associated with human activities, particularly fecal contamination, on the structural variations of bacterial communities and ARG profiles in coastal waters, marker genes of crAssphage were annotated by aligning sequences against the crAssphage genomes (NC_024711.1) downloaded from GenBank using Bowtie2 (v2.4.5) with default settings (Karkman et al., 2019). Phage abundance in each

sample was represented by genome coverage calculated by SAMtools (v1.6) (Karkman et al., 2019).

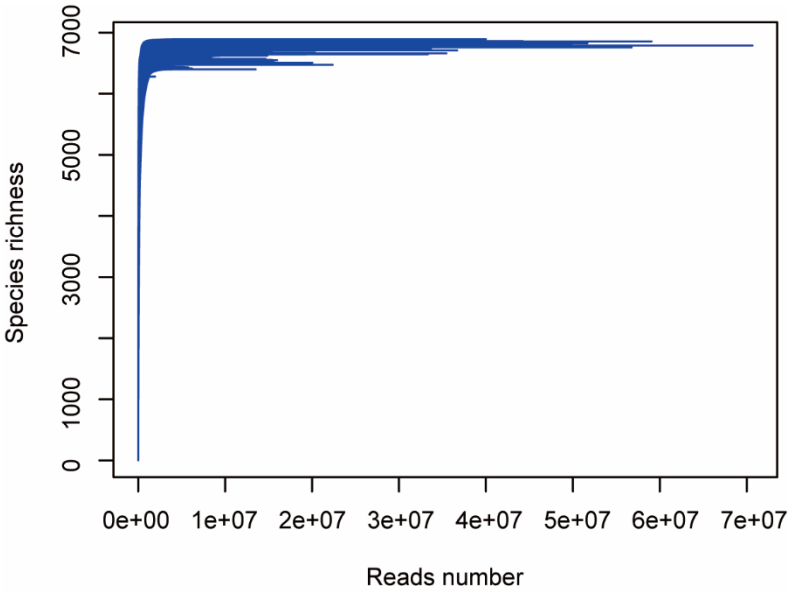

Figure 3-3 Rarefaction curves of bacterial species for oceanic and coastal samples analyzed by the 'vegan' package in R.

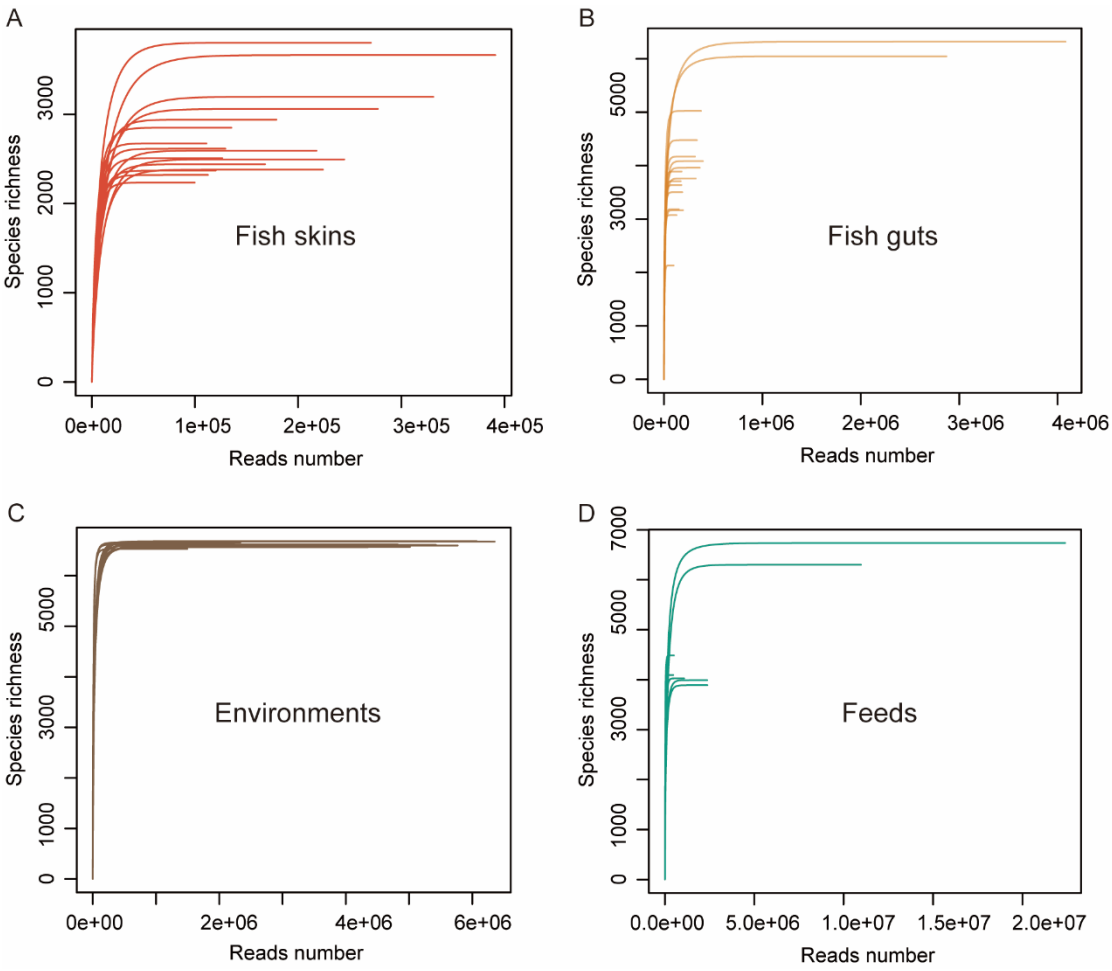

Figure 3-4 Rarefaction curves of bacterial richness in fish skins (A), fish guts(B), environmental samples (C), and feeds (D) analyzed by the 'vegan' package in R.

3.6.3.2 Contig-based ARG hosts and resistance with mobility and pathogenicity

Clean reads from each sample were assembled into contigs of at least 500 bp using Megahit (v1.1.1) with a -k-min 61 parameter (Li et al., 2016). ARG-like open reading frames (ORFs) were identified using the BLASTX mode of DIAMOND (v2.0.13.151) (Buchfink et al., 2015), aligning against the Structured ARG database relevant to each study (SARG v2.2 for coastal investigations; SARG v3.2.1-S for mariculture), with parameters consistent with those of the ARG-OAP pipeline. ORF prediction was first

performed using Prodigal (v2.6.3) with default settings (Hyatt et al., 2010). The relative abundance of each ARG type or subtype was calculated according to the methods described previously (Yin et al., 2023), with the unit adjusted from GeneCopy per Gb (ARG copy of mapped nucleotide bases per Giga bases) in coastal investigations to ARG copies per cell (copy of ARGs normalized against the cell number) in the mariculture study. The equations are shown as follows.

GeneCopy per Gb (Coverage, ×/Gb):

$$\frac{\Sigma_{i=1}^{n} R_{i\ \text{ARG-like ORFs}} \,/\, L_{i\ \text{ARG reference sequence}}}{B \,/\, 10^{9}}$$

ARG copies per cell [copy/cell, here is to apply the mean copies of 30 essential bacterial (or prokaryotic) single-copy marker genes (ESCMGs)]:

$$\frac{\Sigma_{i=1}^{n} R_{i\ \text{ARG-like ORFs}} \,/\, L_{\text{i ARG reference sequence}}}{\frac{1}{30} \times \Sigma_{k=1}^{30} \sum_{j=1}^{m} R_{jk\ \text{marker-like reads}} \,/\, L_{jk\ \text{marker gene}}}$$

$R_{i\ \text{ARG-like ORFs}}$ is the sum of aligned lengths of all reads assigned to the *i*-th ARG reference sequence; $L_{i\ \text{ARG reference sequence}}$ is the nucleotide sequence length of the *i*-th reference sequence in the ARG database; B is the number of bases in the dataset; $R_{jk\ \text{marker like reads}}$ is the sum of aligned lengths of all reads that mapped to the *j*-th reference sequence in the *k*-th marker gene of ESCMGs, where currently 30 genes are usually used (Nayfach & Pollard, 2015); $L_{jk\ \text{marker gene}}$ is the nucleotide sequence length of the *j*-th reference sequence in the *k*-th marker gene cluster of ESCMGs or SCGs.

To assess ARG mobility, we annotated the MGEs that co-occurred with ARGs on the same contig. Transposase, transposon, integrase, integron, recombinase, integrases, and

class 1 integrons (*Int*I1) were detected by aligning ORFs on ARG-carrying contigs (ACCs) against the constructed MGE database (2021/12/16) (Arango-Argoty et al., 2019) using BLASTP mode of DIAMOND (v2.0.13.151) (Buchfink et al., 2015) with thresholds of identity ≥ 80%, query coverage ≥ 75%, and e-value ≤ 10^{-7}. This database consists of 227,640 non-redundant genes retrieved from both the NCBI-NR and the integron-integrase (I-VIP) databases. Its large scale and comprehensive coverage ensure broad representation of functional genes. Plasmids, phages, and phage-plasmid sequences (ACCs identified as both phages and plasmids) were predicted according to the pipelines of PlasFlow (v1.1, with default parameters after filtering the ACCs with a length of no less than 1000 bp) (Krawczyk et al., 2018) and PhaBox (with default parameters after filtering the ACCs with a length of no less than 3000 bp) (Shang et al., 2023). ARGs were considered potentially mobile or transferable if they co-localized with MGEs. ACC taxonomy was annotated using Centrifuge (v1.0.4) with default parameters (Kim et al., 2016). Pathogenic species were classified according to the above-mentioned pathogen list, and ARGs carried by these pathogens were defined as pathogen-associated ARGs. The relative abundance of each ARG host was calculated by normalizing the number of mapped ACCs classified as a bacterium against the total number of bacterial contigs, expressed as parts per million (ppm). The equation is as follows.

Parts per million (ppm):

$$\frac{\Sigma_{i=1}^{n} N_{i\ \text{ARG-carrying contigs}}}{S\ /\ 10^{6}}$$

$N_{i\ \text{ARG-carrying contigs}}$ is the number of ARG-carrying contigs assigned to the *i*-th bacterial

species using Centrifuge (v1.0.4) with default parameters; *S* is the total number of contigs assigned to bacteria using Centrifuge (v1.0.4) with default parameters.

Due to potential loss or incorrect assembly of ARGs during the assembly process, no pathogenic hosts of ARGs were detected in fish samples at the contig level. Consequently, we predicted pathogen-associated ARGs and potential resistant pathogens in fish samples using network analysis based on read-level data, as adopted in previous studies (Feng et al., 2018; Li, Yang, et al., 2015; Zhang et al., 2020). Pathogen-associated ARGs and potential resistant pathogens were identified by the strong associations between ARGs and human pathogens. A Spearman's rank correlation coefficient was considered to be significant based on the thresholds of $r \geq 0.7$ and $p < 0.05$. Only positive correlations were retained for network construction.

3.6.3.3 Metagenome-assembled genome (MAG)-based pathogenic taxonomy and resistance

High-quality MAGs with a minimum completeness of 50% and maximum contamination of 10% were constructed from different coastal and oceanic sites using metaWRAP (v1.3.2), following the established binning and refinement protocols (Uritskiy et al., 2018). Briefly, clean data from coastal seawater samples collected in different seasons were co-assembled by sampling sites using Megahit under the metaWRAP assembly module with default parameters. Oceanic samples were grouped by ocean and co-assembled using the same pipeline. For groups with an excessively

large data volume, the data were partitioned into smaller subsets before co-assembly. The resultant contigs were initially binned using the metaWRAP binning module with three different algorithms: MetaBAT2 (Kang et al., 2019), MaxBin2 (Wu et al., 2016), and CONCOCT (Alneberg et al., 2014). Then, the initial bins were refined and consolidated into high-quality genomes under the refinement module, adhering to the criteria of ≤ 10% contamination and ≥ 50% completeness. Taxonomic classification of the MAGs was performed by aligning them with the Genome Taxonomy Database (GTDBrelease207_v2) using the GTDB-Tk software toolkit (v2.1.0) (Chaumeil et al., 2022). ARGs within MAGs were identified following the same annotation pipeline used for contigs after ORFs prediction by Prodigal (v2.6.3) with default setting (Hyatt et al., 2010).

3.6.3.4 Genome-resolved phylogenetic analysis

To assess the health risks associated with exposure to antimicrobial-resistant human pathogens via seawater recreation and seafood consumption, we investigated the phylogenetic relationships between potential resistant human pathogens in coastal waters and resistant clinical strains implicated in bacterial community–associated infections. Comprehensive genome sets of the targeted pathogenic species, including resistant clinical strains, were downloaded from RefSep using the scripts of ncbi-genome-download (Blin, 2023). A phylogenetic tree was constructed for each pathogenic species by running the infer module integrated in the GTDB-Tk software (v2.1.0) (Chaumeil et al., 2022) and visualized using the iTOL annotation editor

(Letunic & Bork, 2021).

3.6.4 Source tracking

To investigate the source dynamics of fish-associated human pathogens and the resistomes in the mariculture study, we quantified the contributions of putative sources (e.g., seawater, sediments, and feeds) using the fast expectation-maximization microbial source tracking (FEAST) method. The analyses were based on the read counts of annotated pathogens and ARGs according to the multiple sinks protocol with the same source group (Shenhav et al., 2019).

3.7 Isolation of resistant human pathogens from cultured fishes

3.7.1 Bacterial isolation and antimicrobial susceptibility testing

Three adult individuals of each species of similar size were captured from the farm using the same sampling approach described above (Table 3-7). After delivery to the laboratory, the edible tissue (including skins and muscles) of each species was dissected, pooled, and homogenized with sterile equipment. Then, 2.5 g of edible tissue was incubated in 22.5 g of liquid LB broth for 24 h at 37 °C to enrich the bacteria. After enrichment, 10 μL of each sample was spread homogeneously on a solid chromogenic medium plate (CHROMagar^{TM}, Paris, France) for the isolation and differentiation of emerging human pathogens, including *E. coli*, *K. pneumoniae*, *S. aureus*, *Staphylococcus saprophyticus*, *Enterococcus*, *Proteus mirabilis*, and *Citrobacter*. If bacterial colonies grew together after incubation for 24h, then the mixture was diluted

into a series of concentrations (10-, 100- and 1000-fold) according to the colony density and re-inoculated to generate well-separated colonies. Each colony was isolated and submitted to antimicrobial susceptibility testing. The susceptibility of the isolates was examined by the disk-diffusion method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, M100, 28th Edition). A panel of 10 antimicrobial disks was chosen for resistance assessment. The antimicrobials belong to seven drug classes that are commonly used in Hong Kong, including four beta-lactams (ceftriaxone, 30 μg; imipenem, 10 μg; cefotaxime, 30 μg; ampicillin, 10 μg), a fluoroquinolone (ciprofloxacin, 5 μg), a peptide (polymyxin B, 300 IU), a glycopeptide (vancomycin, 30 μg), tetracycline (30 ug), a sulfonamide (trimethoprim-sulfamethoxazole, 1.25-23.75 μg), a MLS antibiotic (erythromycin, 15 μg). *Escherichia coli* ATCC 25929 was employed as the quality control strain. The isolates were classified as susceptible, intermediate, and resistant according to the zone diameter interpretative standards recommendations by CLSI (2019).

Table 3-7 Information on the adult fish collected for surveillance of resistant pathogens.

Species	Feeding behavior	Weight (g)	Length (cm)
Siganus canaliculatus (Park, 1797) (SC)	Herbivore	152.0 ± 8.0	22.5 ± 0.5
Trachinotus blochii (Lacepède, 1801) (TB)	Omnivore	348.3 ± 33.2	30.3 ± 0.6
Epinephelus coioides (Hamilton, 1822) (EC)	Carnivore	1040.7 ± 25.7	42.7 ± 2.1
Epinephelus fuscoguttatus × *Epinephelus lanceolatus (EFL)*	Carnivore	1258 ± 24.6	42.4 ± 0.1

3.7.2 Bacterial identification and whole-genome sequencing

The total genomic DNA of each isolate with resistance was extracted using the QIAamp DNA Mini Kit according to the manufacturer's protocol for isolated bacteria and then sequenced by the Sanger technique. The taxonomy of the isolate was annotated by blasting the sequence on the NCBI website. If the isolate belonged to the target pathogens, then PacBio sequencing was conducted to explore the whole genome. Briefly, after quality control by testing the concentration, integrity, and purity, DNA was sheared using Covaris g-TUBEs to an average size of 7–10 kilobases (kb). The DNA fragments were subjected to the removal of single-strand overhangs, damage repair, and end repair. Subsequently, the repaired DNA from each bacterial strain was ligated with SMRTbell adapters with different barcodes to form SMRTbell sequencing libraries. After purification using 0.45× AMPure PB Beads, the eight sequencing libraries were pooled according to the Microbial Multiplexing Calculator provided by PacBio. The pooled sequencing library was sequenced on the PacBio Sequel platform. The raw sequencing reads were demultiplexed and assembled using SMRT Link software (v7.0). The Assembly (HGAP 4) application with default settings in the SMRT Link software was used for microbial genome assembly.

3.7.3 Phylogenetic analysis and ARG annotation

From the isolation, we detected a strain of *S. aureus* with phenotypic resistance to ciprofloxacin. To further uncover its phylogenetic relationship with clinical strains with resistance, especially MRSA, we performed phylogenetic analysis to cluster the isolate

with 1209 complete genomes downloaded from NCBI RefSep. The genotypic resistance of the isolate and the close genome was annotated using the same pipeline employed in the ARG annotation of coastal pathogenic genomes.

3.8 Statistical analysis

R studio (v4.2.2) was used for data analysis. Differences in bacterial structure, pathogen composition, ARG subtype profile, and ARG host composition across sample groups were assessed using unconstrained principal coordinates analysis (PCoA) based on the Bray – Curtis distance and permutational multivariate analysis of variance (PERMANOVA). Interspecies variations in pathogen composition (at the species level) and ARG profile (at the subtype level) among different cultured fish species were examined through multilevel pairwise comparison performing adonis analysis integrated in the 'vegan' package (Dixon, 2003). Discriminative pathogens and resistomes within groups were identified using the linear discriminant analysis effect size (LEfSe) approach (Chen et al., 2022; Segata et al., 2011). Shared elements, such as pathogenic species, ARG subtypes, potential pathogenic hosts of ARGs, and mobile ARG subtypes between or among different sample groups, were visualized using Venn diagrams. Significant fluctuations of variables in two-group comparisons were tested by running the Wilcoxon rank sum test. Environmental and anthropogenic factors driving the structural changes of coastal microbiomes and resistomes were explored using distance-based redundancy analysis (db-RDA) and partial Mantel tests.

Chater 4 Comparative analysis of coastal and oceanic bacteriomes and resistomes

Marine coastal ecosystems, particularly those adjacent to densely populated cities, are highly vulnerable to stressors arising from anthropogenic activities. As highlighted in Chapter 2, microbial contamination and the prevalence of ARGs have been documented in coastal zones impacted by human activities, including WWTP discharges, contaminated riverine runoffs, mariculture practices, and recreational activities. However, the extent of anthropogenic impacts on coastal bacteriomes and resistomes remains poorly characterized. To address this challenge, we employed advanced NGS and bioinformatics approaches (detailed in Chapter 3) to comprehensively investigate the structure and variations of bacteriomes and resistomes in coastal waters under varying degrees of anthropogenic influence, using surface ocean ecosystems with minimal human impacts as global baselines.

4.1 Shifts in coastal bacteriomes

4.1.1 Bacterial community composition

We performed metagenomic insights into the bacterial taxonomy in human-impacted coastal waters compared with their oceanic counterparts using read-based methods. The dominant phyla, *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Cyanobacteria*, and *Actinobacteria*, were prevalent in both coastal and oceanic systems, together comprising over 95% of the bacterial communities (Figure 4-1). However, *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* were remarkably more abundant in

human-impacted coastal waters compared with their oceanic counterparts (Figure 4-1). There was a significant divergence in bacterial community structure at the species level between the coastal and oceanic systems (Figure 4-2A, PERMANOVA, $R^2 = 0.33$, $p < 0.001$). Further comparison of the core bacterial species between these two systems revealed that 65% of coastal species were different from those in oceanic samples, with a large proportion classified into *Gammaproteobacteria* (Figure 4-3A and B). These results suggest that the differences in bacterial composition may involve alterations in the core bacterial species of coastal waters. LEfSe demonstrated that 497 bacterial species had a discriminative level of relative abundance in human-impacted coastal waters compared to those in surface oceanic systems (linear discriminant analysis [LDA] score > 2, $p < 0.05$), the dominant of which were *Planktomarina temperata*, *Candidatus* Pelagibacter sp. HIMB1321, *Aequoribacter fuscus*, *Synechococcus* sp. LTW-R, *Colwellia* sp. PAMC 20917, *Formosa* sp. Hel1_33_131, *Formosa* sp. Hel3_A1_48, *Marivivens* sp. JLT3646, *Phaeobacter gallaeciensis*, *E. coli*, *Aestuarium zhoushanense*, *Pseudoalteromonas shioyasakiensis*, *K. pneumoniae*, *Pseudoalteromonas arctica*, *Salmonella enterica*, *Pseudomonas* sp. CIP-10, *Phaeobacter inhibens*, *Flavobacterium* sp. Sr18, *Synechococcus* sp. Minos11, and *Pseudoalteromonas agarivorans* (Figure 4-3C). Seventy percent of these dominant species belonged to the core bacterial species in the coastal bacteriome (Figure 4-3C). These findings highlight that shifts in coastal core bacterial species may be associated with anthropogenic influences. Moreover, these anthropogenic impacts may have altered the natural core bacteria in coastal waters and thereby promoted structural changes in coastal bacteriomes. Such processes have

been observed in other human-impacted aquatic systems (Hu et al., 2017) and are also supported by a large number of coastal core bacterial species classified into *Gammaproteobacteria*. This taxa belonging to *Proteobacteria* is known to be dominant in human gut microbiome and less abundant in natural marine bacterial assemblages (Shin et al., 2015). Their increase in ambient environments is recognized as a key indicator of environmental bacterial contamination linked to anthropogenic impacts (Holcomb & Stewart, 2020; Niepceron et al., 2013). Their elevated abundance in coastal core bacterial species reflects the anthropogenic impacts on coastal bacteriomes.

Based on an in-depth investigation of the variations of bacterial communities in human-impacted coastal waters, bacterial phyla exhibited clear seasonal and regional variations (Figure 4-4). Such variations were primarily driven by the changes of the phylum *Proteobacteria* across seasons and regions (Figure 4-4). Notably, seasonal fluctuations were more pronounced than regional differences (Figure 4-4). This pattern aligns with previous findings indicating that seasonality surpassed spatial variability in shaping coastal bacterial assemblages (Wang et al., 2020). Species-level analyses by PCoA clustering further support this conclusion, showing greater separation among seasonal groups compared to regional ones (Figure 4-5A, PERMANOVA, R^2_G = 0.396, p < 0.001). However, there was a large regional difference in bacterial composition in winter, suggesting human disturbances on the variations of coastal bacteriomes. This finding was evidenced by calculating the interaction between group variation and site difference (representing anthropogenic types) using the adonis2 function of

PERMANOVA. Specifically, 32.6% of the coastal bacteriome variations were explained by this interaction (Figure 4-5A, PERMANOVA, $R^2_{G:S} = 0.326$). Additionally, analysis of the discriminative bacterial species revealed that 58.8% (292/497) of these species belong to the *Proteobacteria* phylum, averaging over 50% of the total relative abundance in this group (Figure 4-6A). These species exhibited similar seasonal and regional variation patterns in relative abundance as those of their respective phyla (Figure 4-6B and C). These observations suggest that anthropogenic alterations in *Proteobacteria* may be responsible for the disturbances of the natural dynamics of coastal bacteriomes.

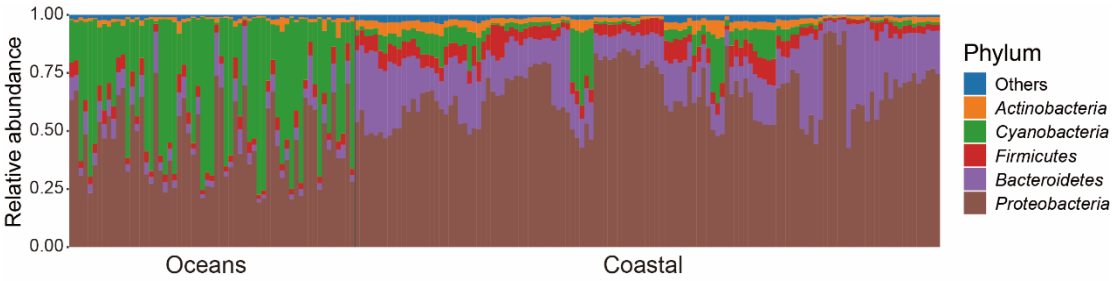

Figure 4-1 Comparison of the bacterial community composition at the phylum level between the oceanic and coastal samples. Both systems share the same predominant phylum (*Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Cyanobacteria*, and *Actinobacteria*). However, human-impacted coastal waters have a significantly increased relative abundance of *Proteobacteria*, *Bacteroidetes*, and *Firmicutes*.

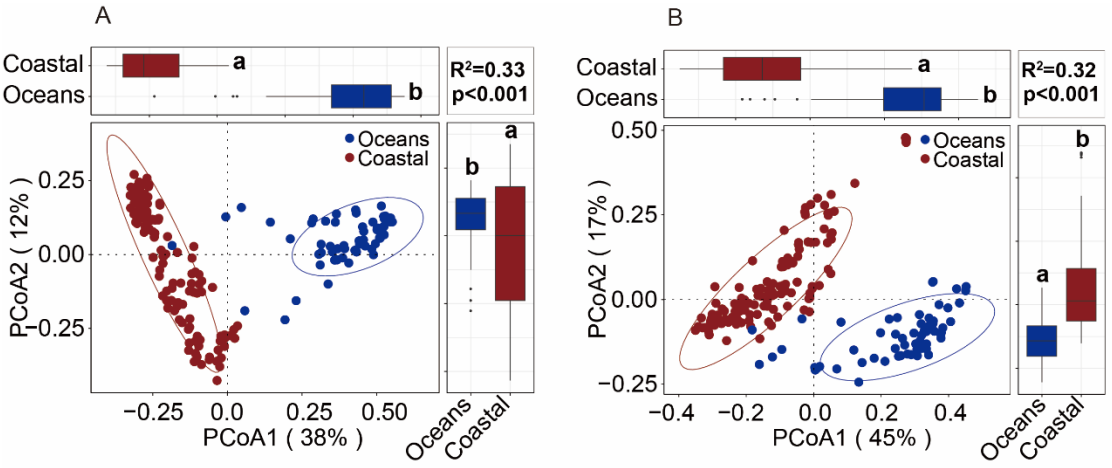

Figure 4-2 Significant differences in the composition of bacterial communities (A) and human pathogens (B) at the species level between human-impacted coastal waters and surface oceans.

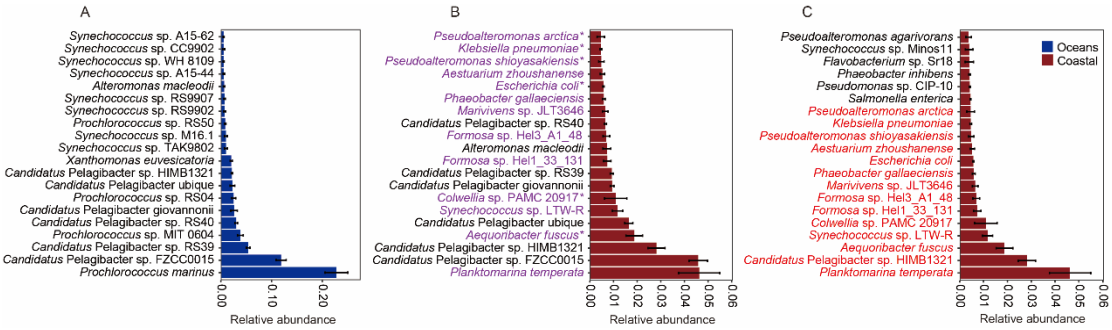

Figure 4-3 Sixty-five percent (13/20, highlighted by purple) of coastal core bacterial species (top 20 species ranked by average relative abundance) (B) were different from those in oceanic samples (A), with a large proportion classified into *Gammaproteobacteria* (*). Seventy percent (14/20, highlighted by red) of the dominant discriminative species were the core bacterial species in coastal bacteriomes (C).

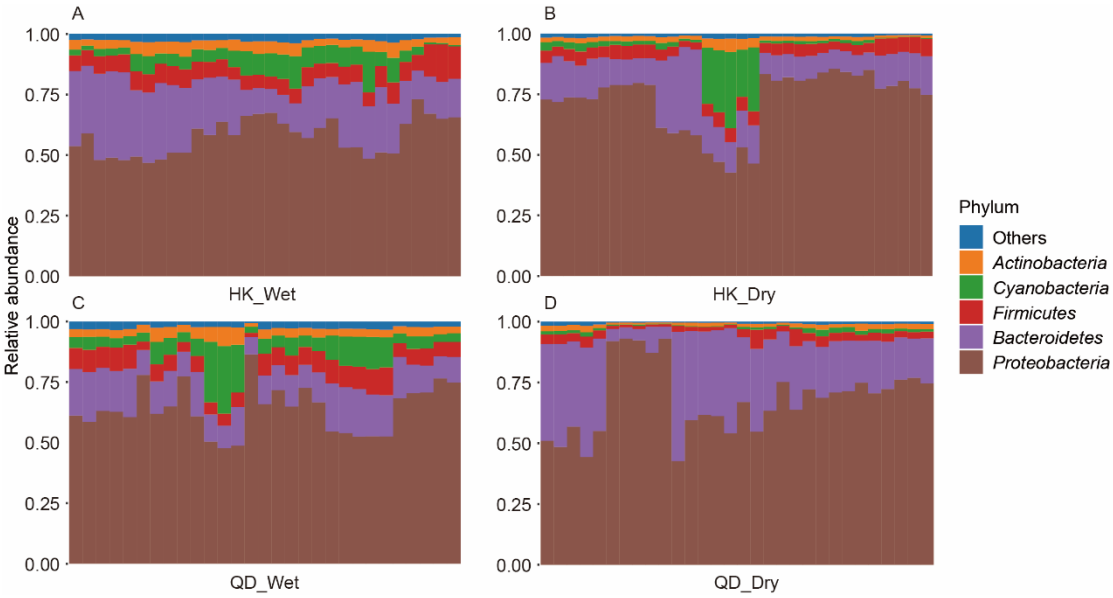

Figure 4-4 Bacterial community composition at the phylum level in different coastal waters across seasons. The bacterial community composition in Hong Kong coastal waters during the wet season (summer) (A) and the dry season (winter) (B). The bacterial community composition in Qingdao coastal waters during the wet season (summer) (C) and the dry season (winter) (D).

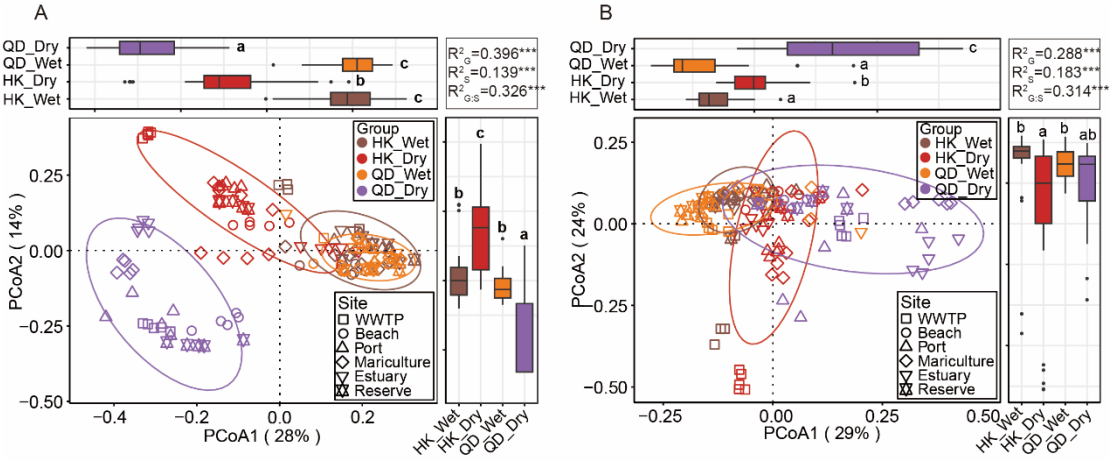

Figure 4-5 Variations in the structural composition of coastal bacteriomes (A) and human pathogens (B) across seasons and regions at the species level.

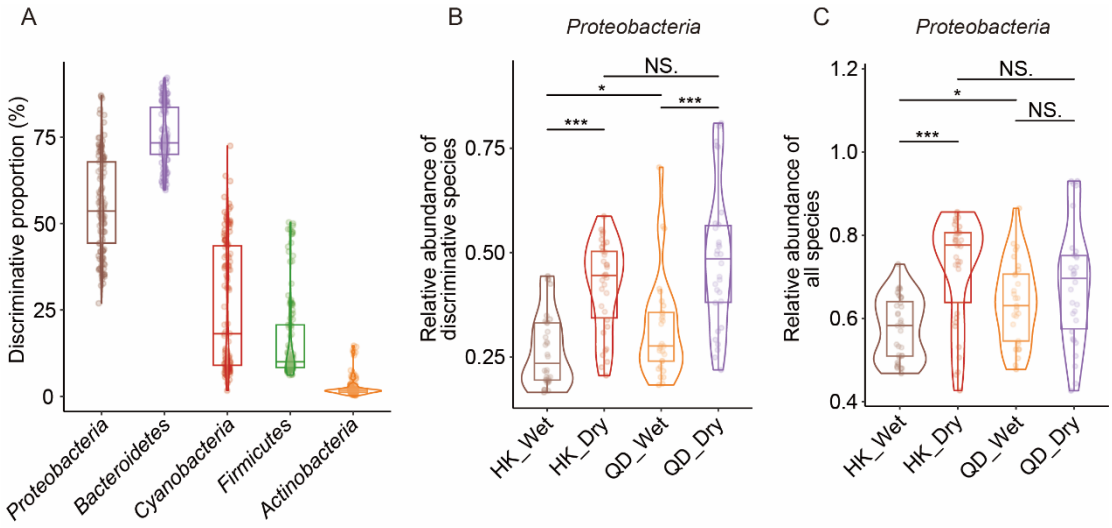

Figure 4-6 Higher abundance of discriminative species that belong to *Proteobacteria* and *Bacteroidetes* (A). Seasonal and regional variations in the relative abundance of discriminative species (B) and total species (C) that belong to *Proteobacteria*.

4.1.2 Biodiversity and human pathogens

We further examined the differences in bacterial diversity and human pathogens between human-impacted coastal waters and oceanic ecosystems based on the above metagenomic-derived bacterial taxonomy. Compared with surface oceans, human-impacted coastal waters presented obviously lower alpha diversity of bacterial species (Figure 4-7A) and significantly higher relative abundance of human pathogens (Figure 4-7B), along with a notable shift in pathogen structure (Figure 4-2B, PERMANOVA, $R^2 = 0.32$, $p < 0.001$). Dominant emerging or foodborne pathogenic species, such as *E. coli, K. pneumoniae, Vibrio cholerae, and V. parahaemolyticus*, exhibited a remarkable increase in relative abundance from surface oceans to coastal waters (LEfSe, LDA score > 2, $p < 0.05$; Figure 4-7C). Indeed, *E. coli* and *K. pneumoniae* were among the core bacteria in coastal waters (Figure 4-3B). These taxa are often indicative of

anthropogenic pollution (Holcomb & Stewart, 2020; Kaper et al., 2004; Ristuccia & Cunha, 1984). The increase in these human-associated pathogens indicates strong anthropogenic impacts on the structural changes in coastal human pathogens. These observations are consistent with previous research documenting the biodiversity loss and pathogen proliferation in other human-affected systems (Xie et al., 2022). Such alterations highlight potential threats to coastal ecosystems and human health induced by anthropogenic activities.

Similarly to bacteriomes, human pathogens also presented significantly higher seasonal variability in composition than regional differences (Figure 4-5B, PERMANOVA, R^2_G = 0.288, $p < 0.001$). Compositional similarity among regional sample groups was notably greater in summer than in winter. Bacterial diversity reached a peak during winter in both coastal regions (Figure 4-8A), coinciding with a marked reduction in pathogen abundance (Figure 4-8B). These observations are consistent with previous research suggesting that coastal bacterial diversity peaks in winter (Gilbert et al., 2012), while marine indigenous pathogens thrive under warmer conditions and in regions of elevated temperature (Belkin & Colwell, 2006). However, variations in pathogen abundance across seasons and regions exhibited relatively less fluctuation than those observed for bacterial diversity, particularly in Hong Kong coastal waters from summer to winter (Figure 4-8A and B), deviating from the patterns typically seen in healthy ecosystems. High microbial diversity can serve as a barrier against pathogen invasion (Berg et al., 2017; Fargione & Tilman, 2005; van Elsas et al., 2012), and an inverse

relationship generally exists between bacterial diversity and pathogen abundance in robust ecosystems (Craft et al., 2022). In this coastal study, this discrepancy may reflect an increasing dominance of human-introduced pathogens within the pathogen assemblage. We found that the discriminative pathogens constituted over 43% of the total pathogen abundance across all samples, with a pronounced rise in the proportion during winter in both regions (Figure 4-8D). The increased proportion of these pathogens may enhance evolutionary resilience and survival of pathogenic bacterial flora to the inhibition of relatively high biodiversity (Amalfitano et al., 2015), leading to the reduced variability in pathogen abundance relative to bacterial diversity. These findings underscore the potential for anthropogenic impacts to drive the proliferation and homogenization of human pathogens across coastal regions and seasons, likely weakening the protective buffer provided by biodiversity. Consistently, anthropogenic impacts explained a large proportion (31.4%) of the variance in pathogen composition (Figure 4-5B, PERMANOVA, $R^2_{G:S}$ = 0.314), and the relative abundance of discriminative pathogens across seasons and regions (particularly in Hong Kong coastal waters across seasons; Figure 4-8C) fluctuated less than that of all pathogenic species (Figure 4-8B). Thus, the elevated presence of human-associated pathogens raised potential risks for increased human exposure, accelerated disease outbreak cycles, and amplified public health threats.

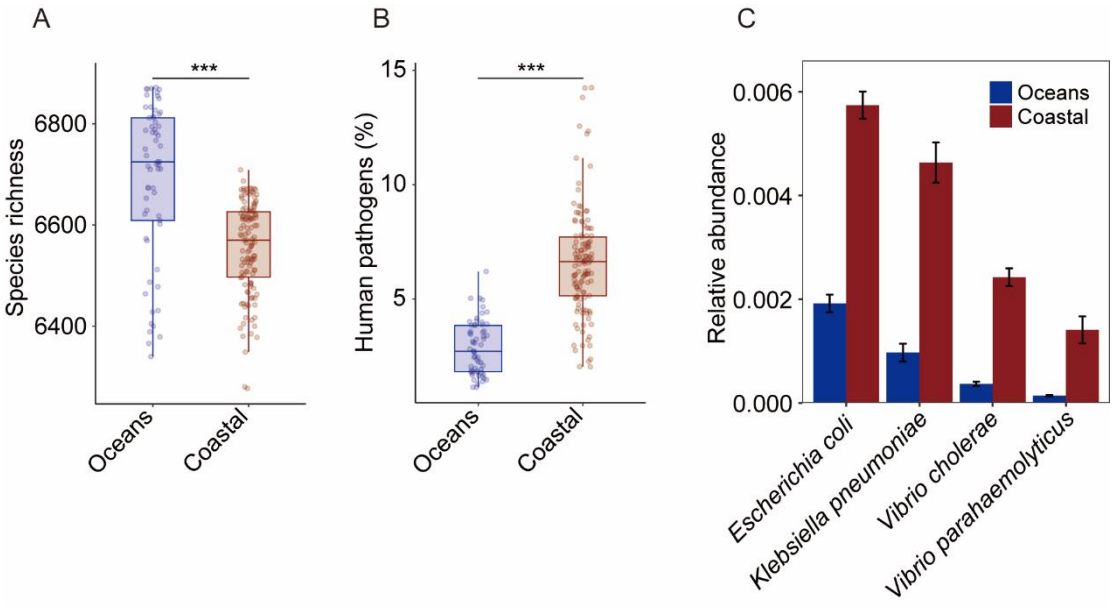

Figure 4-7 Compared with surface oceans, human-impacted coastal waters obviously developed lower bacterial diversity (A) but higher relative abundance of emerging or opportunistic human pathogens (B), especially the emerging and foodborne ones (C).

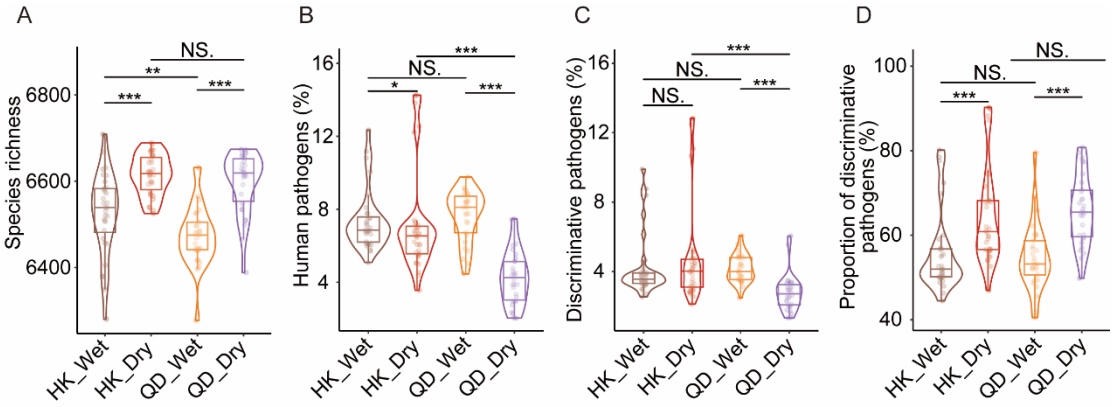

Figure 4-8 Seasonal and regional variations in bacterial diversity (A), human pathogen abundance (B), discriminative pathogen abundance (C), and discriminative pathogen proportions (accounting for the total human pathogen abundance) (D) in coastal waters.

4.2 Changes in coastal resistomes

4.2.1 Profile, diversity, abundance, and health-risk subtype

ARGs in seawater samples from human-impacted coastal waters and oceanic ecosystems were annotated using read-based metagenomic methods. The results show that the ARG profile in human-impacted coastal waters was remarkably distinct from that in surface oceans (Figure 4-9). PCoA based on the Bray–Curtis distance revealed a clear separation between coastal and oceanic samples at the subtype level, indicating a significant difference in the resistome structure between these two environments (Figure 4-10A, PERMANOVA, $R^2 = 0.19$, $p < 0.001$). Human-impacted coastal waters harbored significantly higher diversity and relative abundance of ARG subtypes (Figure 4-10B and C), with core ones (top 20 ranked by the average relative abundance) exhibiting a 35% difference from those in surface oceans (Figure 4-11). These alterations may be due to the notable enrichment of dominant ARGs encoding resistance to multiple drugs, MLS antibiotics, bacitracin, kasugamycin, beta-lactams, trimethoprim, polymyxin, aminoglycosides, and quinolones in coastal waters (Figure 4-12A), which are key prescription human and animal medicine classes (Kemper, 2008; Zanichelli et al., 2023). Based on a critical review, ARG contamination in estuarine environments has been linked to anthropogenic use of antibiotics for human and animal treatments in coastal regions. Such human activities can directly introduce selection agents and ARGs themselves into coastal environments and result in the corresponding rise of antibiotic resistomes (Zheng et al., 2021). Our observation of human-associated ARGs in human-impacted coastal waters reinforces this finding and further indicates

that the expansive influences of land-based antibiotic usage could alter the natural structure of coastal resistomes. Moreover, anthropogenic impacts may have escalated the health risks of resistomes in coastal waters. The discriminative ARGs in coastal waters (LEfSe, LDA score > 2, $p < 0.05$) belonging to the 'current threats (Rank I)' and 'future threats (Rank II)' ARG families (Zhang et al., 2021) were 10-fold higher than those in surface oceans (Figure 4-12B), over 50% of which were high-risk genes highlighted by the World Health Organization (WHO) and previous studies (Zhang et al., 2021), including *lnuA*, *dfrA1*, *dfrA14*, *dfrA17*, *ermC*, *mdtE*, *aph(6)-I*, *aac(6')-I*, *vanY*, *lnuB*, *mdtL*, *aadE*, *ermB*, *msrA*, *catB*, *qnrS*, and *TolC* (Figure 4-12C). These genes confer resistance to aminoglycosides, MLS antibiotics, quinolones, and trimethoprim, which are also key human medicines listed in the access and watch groups of AWaRe (Access, Watch, Reserve) antibiotics (WHO, 2021).

Differing from the variations of coastal bacteriomes, the ARG profiles at the drug class level remained relatively stable across the seasons and regions (Figure 4-13). Nonetheless, the richness and relative abundance of the ARG subtypes increased from summer to winter in both coastal waters (Figure 4-14A and B). Specifically, we observed a regional increase in ARG richness from Hong Kong to Qingdao coastal waters during winter, with an unexpected inverse trend in ARG abundance (Figure 4-14A and B). In winter, Hong Kong coastal waters harbored the highest relative abundance of ARGs. Detailed resistome composition analysis at the subtype level showed high structural differences between seasonal groups and winter region groups

(Figure 4-15A, PERMANOVA, $R^2_G = 0.325$, $p < 0.001$). The sharp increase in human-associated ARGs appears to drive the variations of coastal resistomes, as shown by the congruent seasonal and regional patterns in the richness and relative abundance between these genes and total resistomes (Figures 4-16 and 4-17). It is also evident that the top 50 discriminative ARG subtypes (which together constituted over 88% of the total average relative abundance) displaying the seasonal and regional trends paralleling those of coastal resistomes overall belonged to these human-associated ARGs (Figure 4-14C and D). These results highlight the profound effects of human activities in reshaping these variations. This finding is further supported by the interaction between group variation and site difference (indicative of anthropogenic impacts), which explained 37.5% of the resistome dissimilarity among the samples (Figure 4-15A, PERMANOVA, $R^2_{G:S} = 0.375$). Interestingly, the richness and relative abundance of discriminative high-risk ARG subtypes (which averaged over 55% of total richness and 88% of total relative abundance among the high-risk ARG subtypes) showed similar patterns with the total high-risk ones (Figure 4-18A–F). Collectively, these findings indicate that anthropogenic impacts have disrupted the natural variations of coastal resistomes, particularly high-risk ARGs, elevating potential antibiotic resistance exposure hazards for coastal populations.

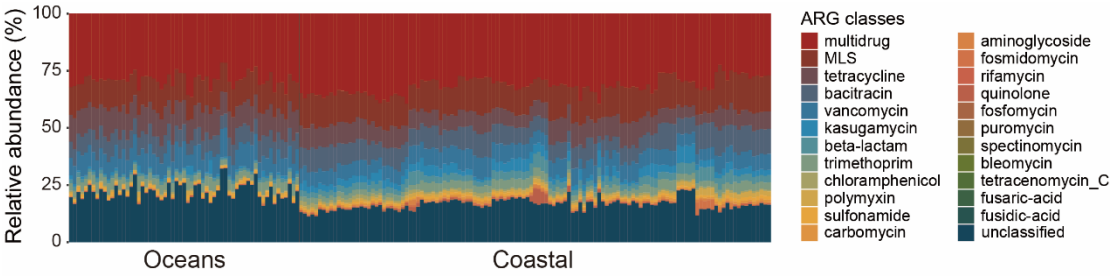

Figure 4-9 Coastal and oceanic seawater exhibited different profiles at the class level. ARG, antibiotic resistance gene; MLS, macrolide–lincosamide–streptogramin.

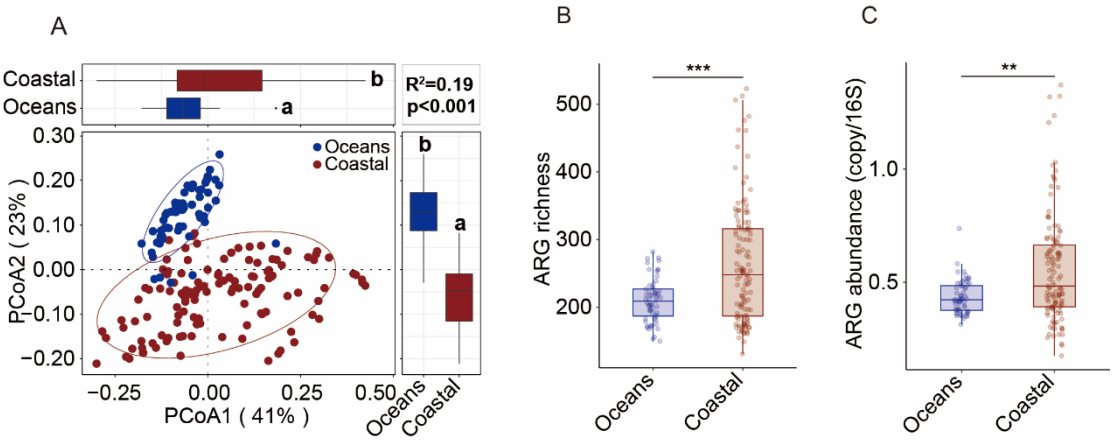

Figure 4-10 Principal coordinates analysis based on the Bray–Curtis distance revealed a significant difference in the structure of resistomes between coastal and oceanic samples at the subtype level (A). Human-impacted coastal waters harbored significantly higher richness (B) and relative abundance (C) of antibiotic resistance gene (ARG) subtypes.

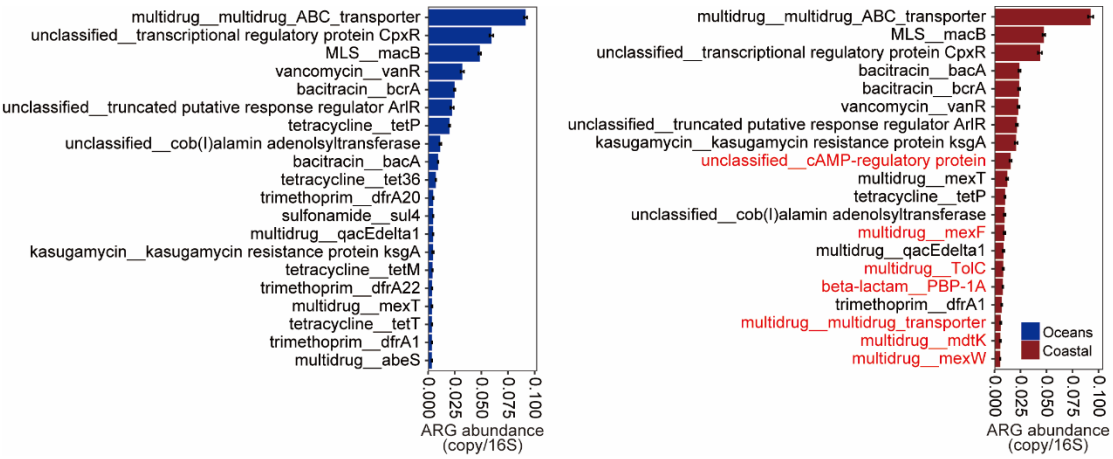

Figure 4-11 Thirty-five percent (7/20) of core subtypes (red) within coastal resistomes were different from those identified in surface oceans. ARG, antibiotic resistance gene; MLS, macrolide–lincosamide–streptogramin.

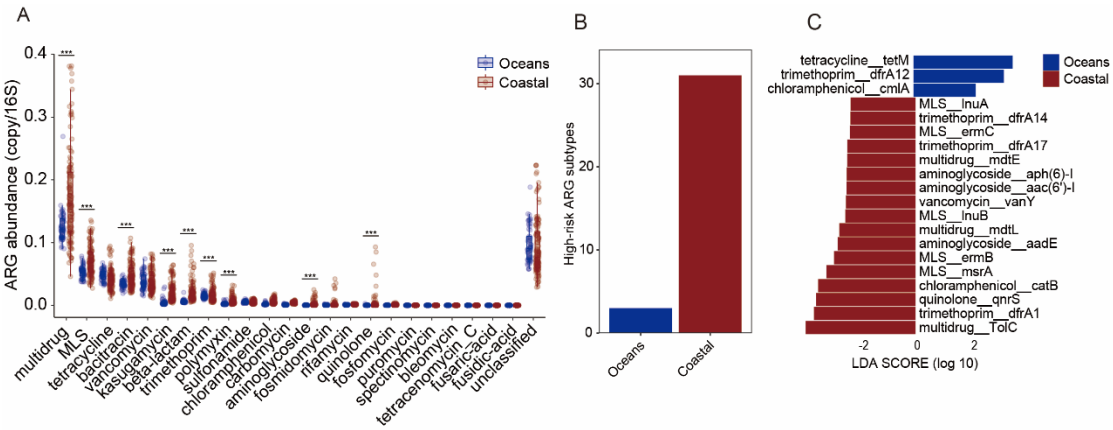

Figure 4-12 There was notable enrichment of dominant antibiotic resistance genes (ARGs) encoding resistance to multiple drugs, macrolide–lincosamide–streptogramin (MLS) antibiotics, bacitracin, kasugamycin, beta-lactams, trimethoprim, polymyxins, aminoglycosides, and quinolones in coastal waters (A). The Rank I and II ARG subtypes were 10-fold higher in human-impacted coastal waters compared with surface oceans (B), over 50% of which were high-risk genes highlighted by the World Health Organization and literature reviews (C).

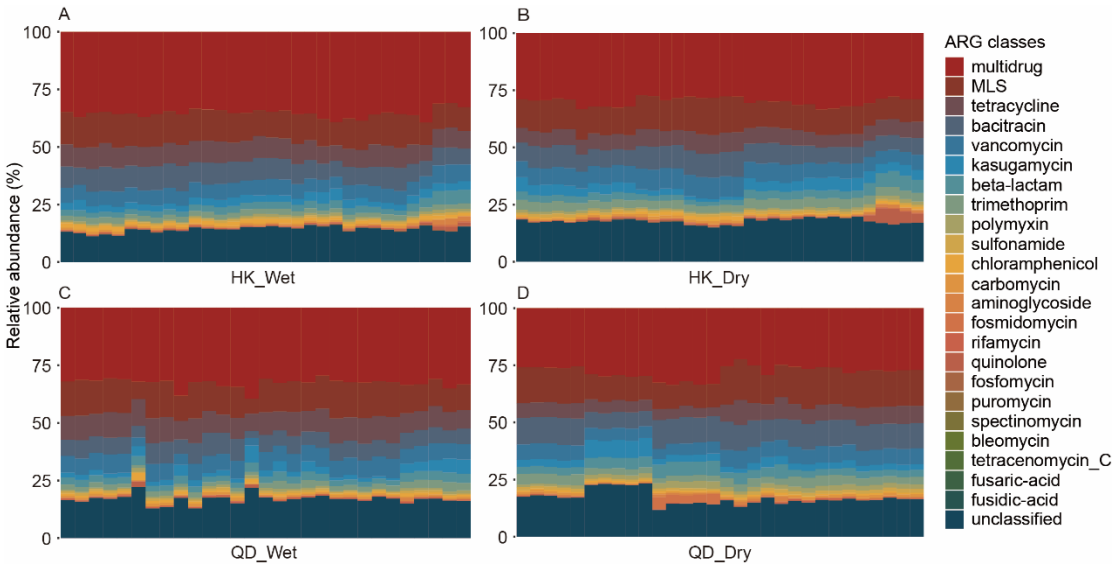

Figure 4-13 Coastal antibiotic resistance gene (ARG) profiles at the drug class level remained relatively stable across seasons and regions. The ARG profiles in Hong Kong coastal waters during summer (A) and winter (B). The ARG profiles in Qingdao coastal waters during summer (C) and winter (D). MLS, macrolide–lincosamide–streptogramin.

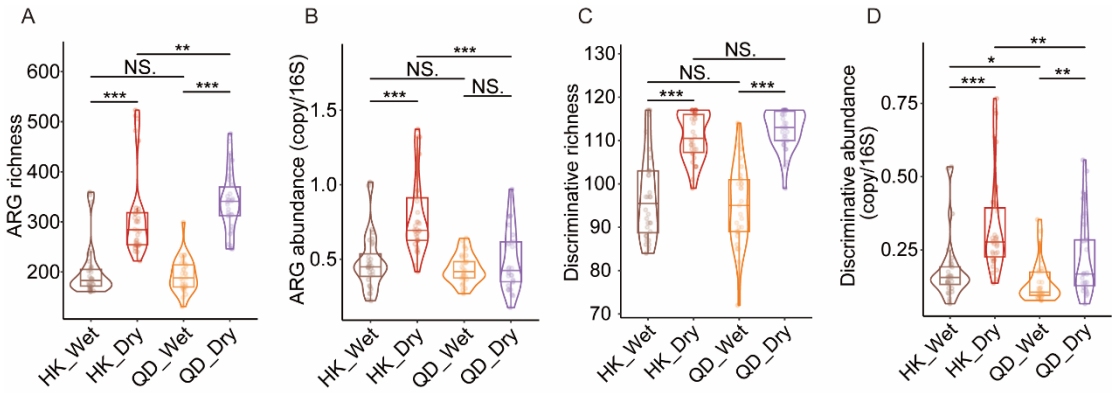

Figure 4-14 The richness (A) and relative abundance (B) of antibiotic resistance gene (ARG) subtypes increased from summer to winter in both coastal waters, while there was only a regional increase in ARG richness (A) from Hong Kong to Qingdao coastal waters during winter along with an unexpected decrease in ARG abundance (B). There were similar seasonal and regional trends in the dominance of the top 50 discriminative

ARG subtypes, which together constituted over 88% of the total average relative abundance (C and D).

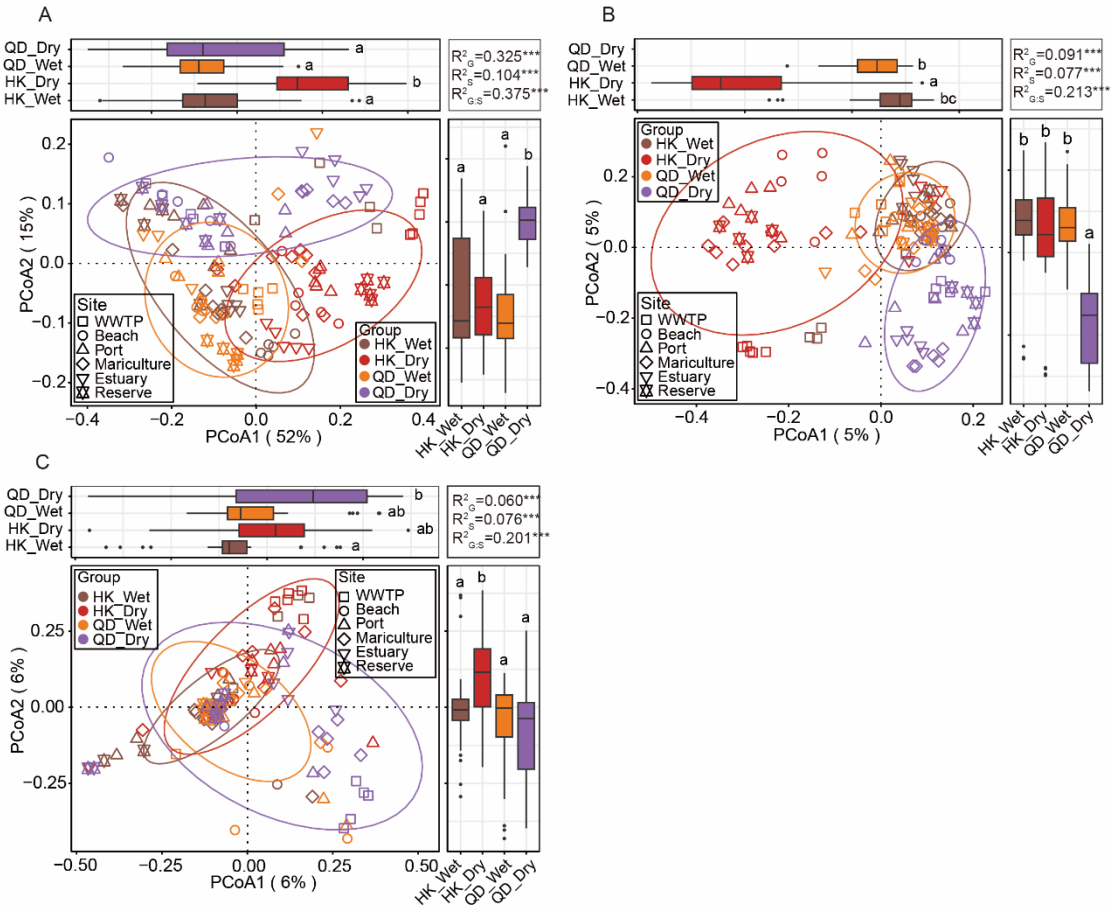

Figure 4-15 Seasonal and regional variations in the composition of coastal resistomes (A), antibiotic resistance gene (ARG) hosts (B), and pathogenic hosts (C) at the species level.

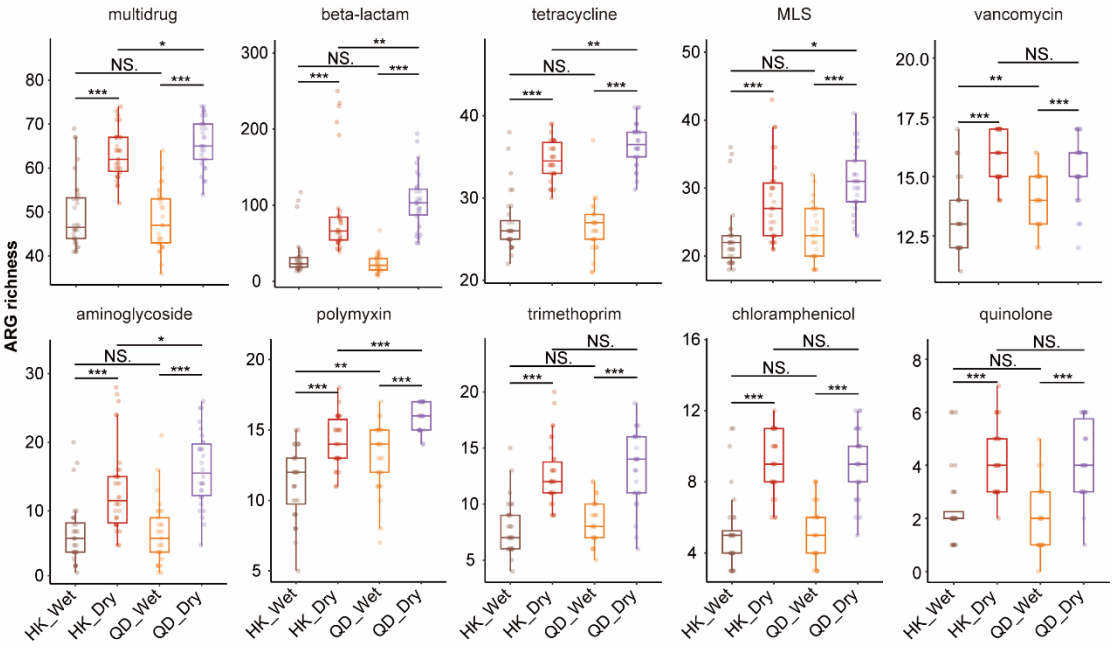

Figure 4-16 Human-associated antibiotic resistance genes (ARGs) among the dominant resistomes conferring resistance to multiple drugs, beta-lactams, tetracyclines, macrolide – lincosamide – streptogramin (MLS) antibiotics, aminoglycosides, polymyxins, and trimethoprim exhibited similar seasonal and regional richness patterns as those observed in total ARG richness.

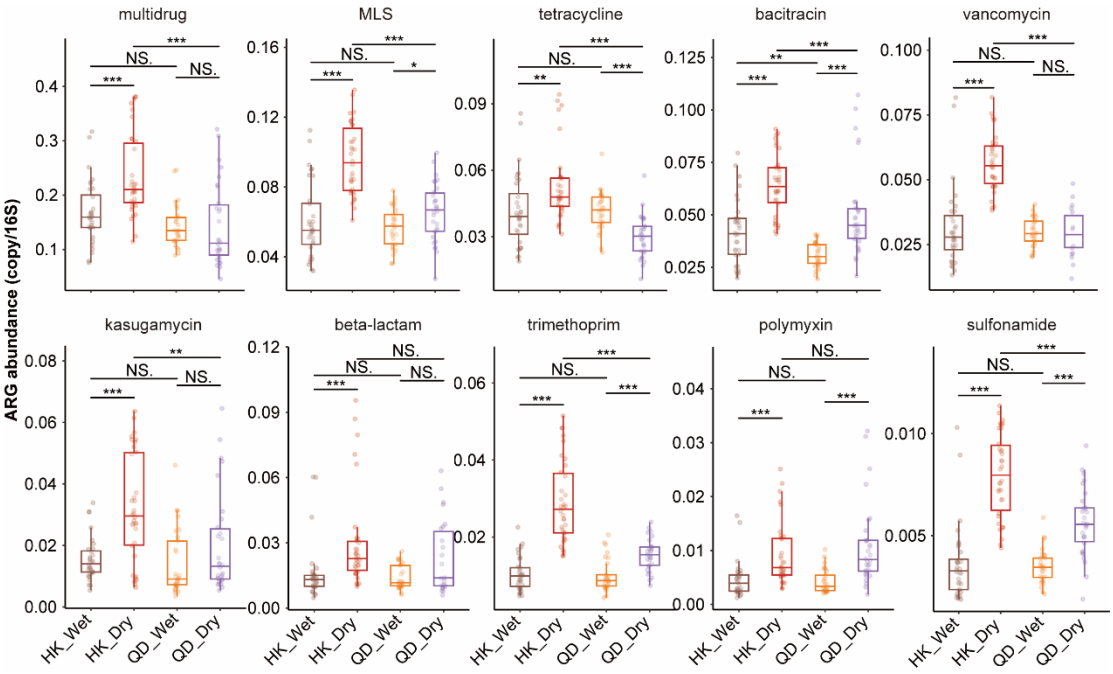

Figure 4-17 Human-associated antibiotic resistance genes (ARGs) among the dominant resistomes conferring resistance to macrolide – lincosamide – streptogramin (MLS) antibiotics, bacitracin, kasugamycin, beta-lactams, and trimethoprim showed similar seasonal and regional abundance patterns as those observed in total ARG abundance.

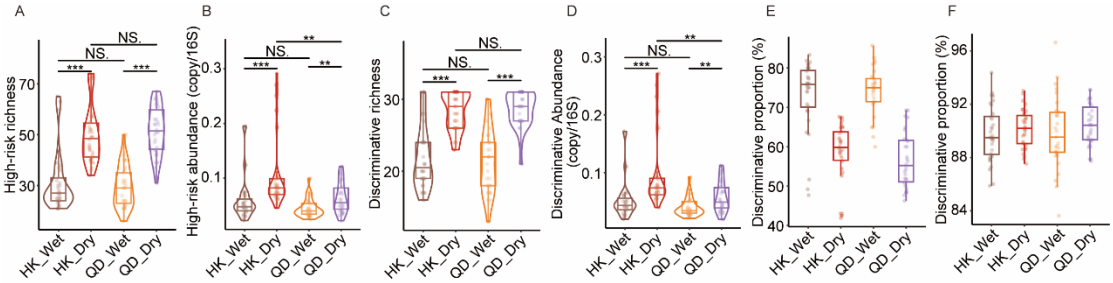

Figure 4-18 The richness (A) and relative abundance (B) of high-risk antibiotic resistance gene (ARG) subtypes increased from summer to winter in both coastal waters, but regional variations only displayed in relative abundance with a significant reduction during winter. Similar seasonal and regional trends in the richness (C) and relative abundance (D) of discriminative subtypes were found in both coastal waters. The

average proportion of the richness (E) and relative abundance (F) of discriminative subtypes accounted for more than 55% and 88%, respectively, in all sample groups.

4.2.2 ARG mobility

We further assessed the mobility of ARGs through metagenomic assembly by identifying those co-occurring with MGEs on the same contig, which were defined as mobile ARGs. The composition of mobile ARG subtypes varied markedly between coastal and oceanic systems (Figure 4-19A, PERMANOVA, $R^2 = 0.08$, $p < 0.001$), with both diversity and relative abundance of these genes increasing significantly from surface oceans to coastal environments (Figure 4-19B and C). This may be associated with the increase in mobile ARGs conferring resistance to critical human and veterinary medicines, which dominated the mobile resistomes in coastal waters (Figure 4-20). The enhanced mobility of these human-associated ARGs may contribute to the structural shifts and abundance enrichment of resistomes in coastal environments. Various human-associated high-risk ARGs were included in mobile subtypes shared by non-pathogenic and pathogenic hosts (Figure 4-21A and Table 4-1) and mobile pathogen-associated ARG subtypes (Figure 4-21B and Table 4-2) in coastal waters. Taken together, these results suggest that coastal ARGs, especially human-associated genes, exhibit strong transferability, even from non-pathogenic bacteria to human pathogens or among resistant human pathogens. This finding aligns with previous studies that have demonstrated a tendency for human-associated ARGs to disseminate rapidly among environmental bacteria, reaching clinically significant species under selective pressure

and primarily transmitted via plasmid vectors (Castañeda-Barba et al., 2024). Our analysis also revealed that plasmid-associated ARGs represent the largest component of mobile resistomes in coastal waters (Figure 4-21C), with a significantly elevated relative abundance compared with oceanic waters (Figure 4-21D). These findings underscore the pivotal role of plasmids as vectors in coastal ARG transmission, potentially accelerating the acquisition of resistance traits by bacteria and human pathogens and supporting the proliferation of coastal resistomes, ARG hosts, and resistant pathogens.

Further analysis of seasonal and regional variations of mobile ARG variables in human-impacted coastal waters showed a seasonal increase in the richness and host abundance of mobile ARGs from summer to winter, particularly in Hong Kong coastal waters (Figure 4-22A–C). Hence, ARG transfer to bacteria was elevated during winter in both coastal waters, particularly in Hong Kong coastal waters. Previous studies have suggested an inverse relationship between bacterial diversity and ARG transmission in natural environments (Chen et al., 2019). However, our findings showed similar seasonal increases between these variables in both coastal waters. This disparity demonstrated a great influence of human activities on the natural seasonal and regional variations of ARG mobility in coastal environments. Such a finding is further supported by the large portion (26.6%) of seasonal and regional variations in mobile resistome composition explained by anthropogenic impacts (Figure 4-23A, PERMANOVA, $R^2_{G:S}$ = 0.266). In fact, the seasonal increase in ARG mobility was related to the enhanced

transfer potential of human-associated ARGs during winter, as these ARGs dominated the coastal mobile resistomes and exhibited a higher prevalence during winter, such as kasugamycin, beta-lactams, and aminoglycosides in Hong Kong coastal waters, and MLS antibiotics, bacitracin, kasugamycin, and beta-lactams in Qingdao coastal waters (Figure 4-23B). The winter increase in the mobility of these ARGs may explain, to some extent, the elevation in the relative abundance of ARGs and their hosts during this season in both coastal waters, particularly in Hong Kong coastal waters. These results suggest that the enhancement of ARG mobility had a marked role in altering the seasonal and regional variations of resistomes and host bacteria in coastal waters. Meanwhile, the higher mobility of pathogen-associated ARGs in Qingdao compared with Hong Kong during winter indicates that the ARG spread to pathogens was more frequent in Qingdao during this season (Figure 4-22C and D). This process may reduce the regional differentiation of resistant pathogens during winter. On the other hand, we observed a large difference in the regional composition of mobile ARGs in winter, alongside significant variations across seasonal groups in both coastal waters (Figure 4-23A, PERMANOVA, $R^2_G = 0.098$, $p < 0.001$). These seasonal and regional patterns of mobile ARG composition likely contributed to the great divergence in the structure of coastal resistomes, ARG hosts, and resistant pathogens across seasons in both regions and between regions in winter.

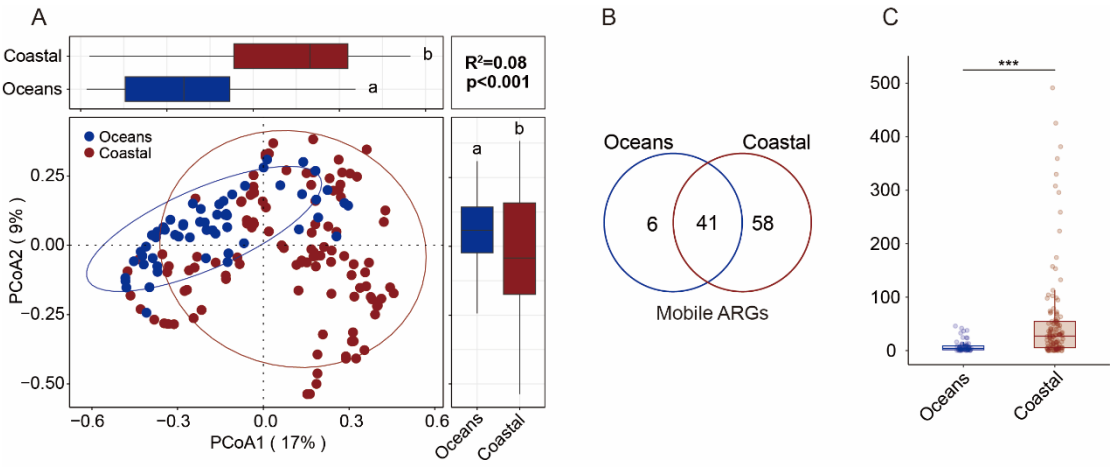

Figure 4-19 Mobile antibiotic resistance gene (ARG) subtypes exhibited a significant compositional divergence between coastal and oceanic samples (A), with both diversity (B) and relative abundance (C) significantly increasing from surface oceans to coastal waters.

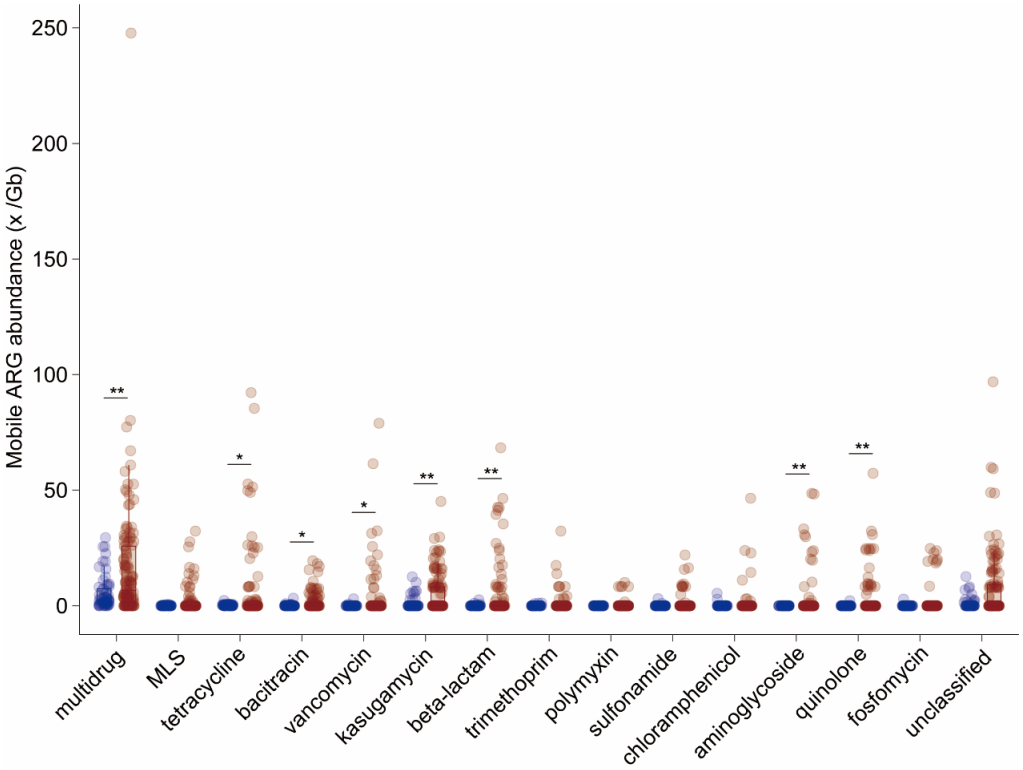

Figure 4-20 Mobile antibiotic resistance genes (ARGs) conferring resistance to key human and animal medicines presented an increase from surface oceans to coastal

waters and dominated coastal mobile resistomes. MLS, macrolide–lincosamide–streptogramin.

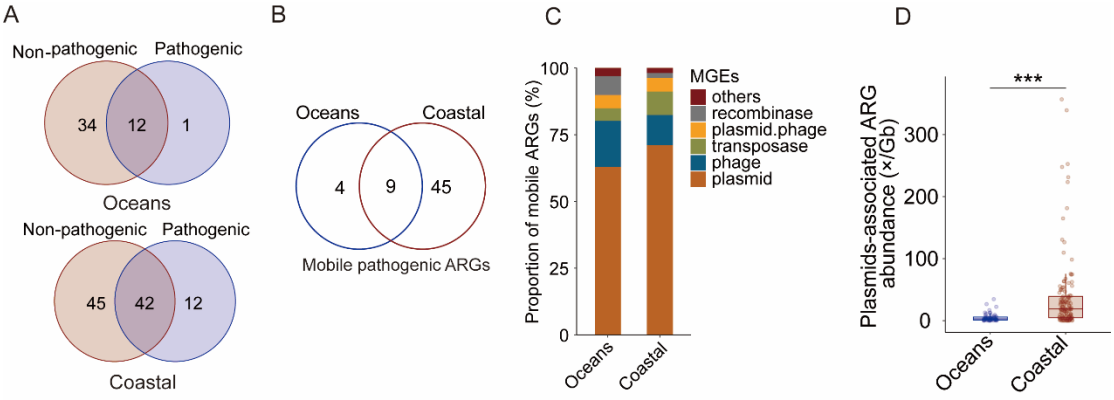

Figure 4-21 More mobile subtypes were shared by non-pathogenic and pathogenic hosts in coastal waters (A). There was higher diversity of mobile pathogen-associated antibiotic resistance gene (ARG) subtypes in coastal waters (B). Plasmid-associated ARGs constituted the highest proportion of coastal mobile resistomes (C), with significantly higher relative abundance compared with surface oceans (D).

Table 4-1 High-risk antibiotic resistance genes (ARGs) are shared by non-pathogenic and pathogenic hosts.

Mobile subtypes shared by non-pathogenic and pathogenic hosts	Location	Rank	High-risk*
Aminoglycoside__aac(6')-I	Coastal	I	Yes
Aminoglycoside__aph(6)-I	Coastal	I	Yes
Chloramphenicol__catB	Coastal	I	Yes
MLS__ermB	Coastal	I	Yes
Multidrug__TolC	Coastal	I	Yes
Quinolone__qnrA	Coastal	I	Yes
Quinolone__qnrS	Coastal	I	Yes
Tetracycline__tetM	Coastal	I	Yes
Multidrug__TolC	Oceans	I	Yes

MLS, macrolide–lincosamide–streptogramin.

Table 4-2 High-risk pathogen-associated antibiotic resistance genes (ARGs) with mobility in coastal waters.

Mobile pathogen-associated ARGs	Location	Rank	High-risk*
Aminoglycoside__aac(6')-I	Coastal	I	Yes
Aminoglycoside__aph(6)-I	Coastal	I	Yes
Beta-lactam__VEB-3	Coastal	I	Yes
Beta-lactam__VIM-2	Coastal	I	Yes
Chloramphenicol__catB	Coastal	I	Yes
MLS__ermB	Coastal	I	Yes
MLS__ermC	Coastal	I	Yes
Quinolone__qnrA	Coastal	I	Yes
Quinolone__qnrS	Coastal	I	Yes
Tetracycline__tetM	Coastal	I	Yes
Trimethoprim__dfrA15	Coastal	I	Yes
Trimethoprim__dfrA17	Coastal	I	Yes

* High-risk ARG families identified by World Health Organization and literature reviews. MLS, macrolide–lincosamide–streptogramin.

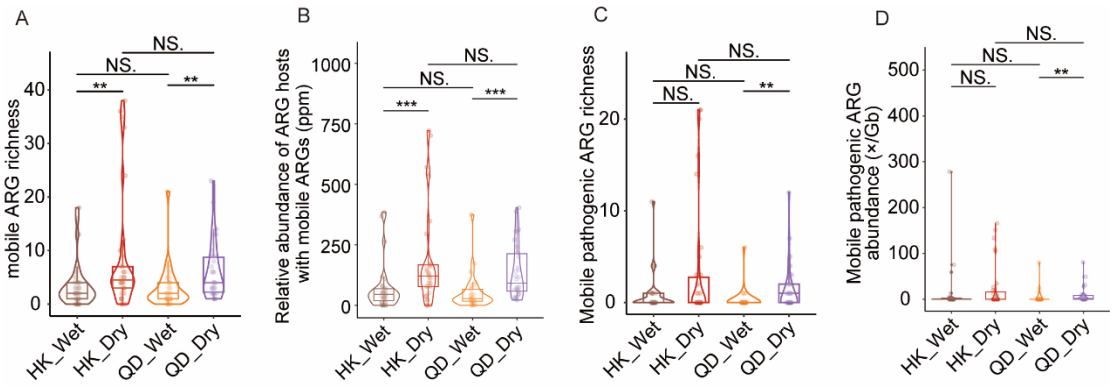

Figure 4-22 There was a seasonal increase in mobile antibiotic resistance gene (ARG) richness (A) and host abundance (B) from summer to winter, particularly in Hong Kong coastal waters. Mobile pathogenic ARG richness (C) and abundance (D) increased from summer to winter in both coastal waters, particularly in Qingdao coastal waters.

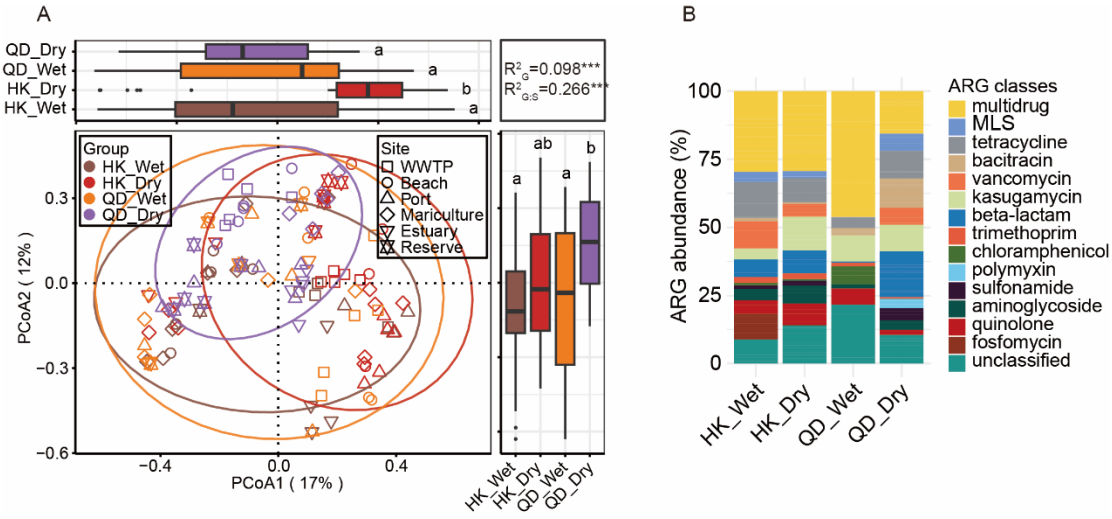

Figure 4-23 Seasonal and regional variations in the composition of coastal mobile antibiotic resistance genes (ARGs) (A). Human-associated ARG types among the dominant resistomes, such as kasugamycin, beta-lactams, and aminoglycosides in Hong Kong coastal waters, and to macrolide–lincosamide–streptogramin (MLS) antibiotics, bacitracin, kasugamycin, and beta-lactams in Qingdao coastal waters, exhibited a higher proportion in winter (B).

4.2.3 Host associations: abundance, composition, and pathogenicity

ARG hosts were identified based on taxonomic classification of metagenome-assembled ARG-carrying contigs. Taxonomic analysis revealed that both oceanic and coastal ARG hosts were predominantly assigned to the same phyla, including *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Cyanobacteria*, and *Actinobacteria*. Notably, *Proteobacteria* exhibited an obvious increase in relative abundance in human-impacted coastal waters (Figure 4-24). Coastal waters developed a significantly different structure of ARG hosts (Figure 4-25A, PERMANOVA, $R^2 = 0.042$, $p < 0.001$) and pathogenic hosts (Figure 4-25D, PERMANOVA, $R^2 = 0.029$, $p < 0.001$),

characterized by a broader range (Figure 4-25B and E) and greater relative abundance (Figure 4-25C and F). These hosts harbored a significantly distinct resistome profile from that of oceanic counterparts (Figure 4-26A, PERMANOVA, $R^2 = 0.16$, $p < 0.001$), with a notable enrichment of the above-mentioned human-associated ARGs in coastal environments (Figure 4-26B). We observed a similar trend in coastal pathogenic hosts (Figure 4-26C, PERMANOVA, $R^2 = 0.07$, $p < 0.001$; Figure 4-26D) with an increased prevalence of high-risk ARGs, including *aac(6')-I*, *aph(6)-I*, *VIM-2*, *VEB-3*, *catB*, *tetM*, *qnrA*, *qnrS*, *dfrA15*, *drfA17*, *ermB*, and *ermC* (Table 4-2). Seventy-five percent of the core resistant bacteria showed changes in coastal waters, with unique species mostly belonging to the *Gammaproteobacteria* phylum (Figure 4-27). Notably, 84 % of coastal human pathogens carrying mobile ARGs were specific to coastal ecosystems (Figure 4-28A), including WHO-designated priority emerging pathogenic species (Tacconelli et al., 2018) and those regarded as foodborne pathogens, such as *Acinetobacter baumannii*, *Enterococcus faecium*, *Helicobacter pylori*, *Campylobacter coli*, *S. pneumoniae*, *Haemophilus influenzae*, *K. pneumoniae*, *E. coli*, *S. aureus*, *Clostridium botulinum*, *Clostridium perfringens*, *Listeria monocytogenes*, *V. parahaemolyticus*, *Vibrio vulnificus*, and *Aeromonas caviae* (Figure 4-28B). These results indicate that anthropogenically enhanced transmission of ARGs and anthropogenically introduced resistant bacteria may have promoted structural shifts and the increased level of resistant pathogens in coastal ARG host populations.

In both Hong Kong and Qingdao coastal waters, the relative abundance of ARG hosts

and resistant pathogens increased seasonally from summer to winter (Figure 4-29A and B). However, we only observed a regional decline in ARG hosts during winter (Figure 4-29A and B). There were similar seasonal variations in the composition of these biological groups, but an unexpected regional divergence emerged in winter (Figure 4-15B, PERMANOVA, R^2_G = 0.091, p < 0.001; Figure 4-15C, PERMANOVA, R^2_G = 0.060, p < 0.001). Notably, the seasonal and regional patterns in the relative abundance of these biological groups diverged from those observed in biodiversity, ARGs, and human pathogens. High bacterial diversity is known to be an inhibitor for the spread and proliferation of ARGs, ARG hosts, and resistant pathogens in natural environments (Bagra et al., 2023; Chen et al., 2019; Uli et al., 2023). The unexpected divergence reflects anthropogenic footprints on the natural seasonal and regional variations of coastal resistant bacteria, as also evidenced by the observation that the interaction between group variation and site difference contributed to 21.3% and 20.1% of the compositional dissimilarity in these elements (Figure 4-15B, PERMANOVA, $R^2_{G:S}$ = 0.213; Figure 4-15C, PERMANOVA, $R^2_{G:S}$ = 0.201). These findings highlight the potential for human activities to augment the seasonal proliferation of ARG hosts and resistant pathogens, as well as their resultant health risks during winter. According to the above discussion, ARG transmission may be an important factor in shaping the seasonal and regional patterns of ARG hosts and resistant pathogens in human-impacted coastal waters.

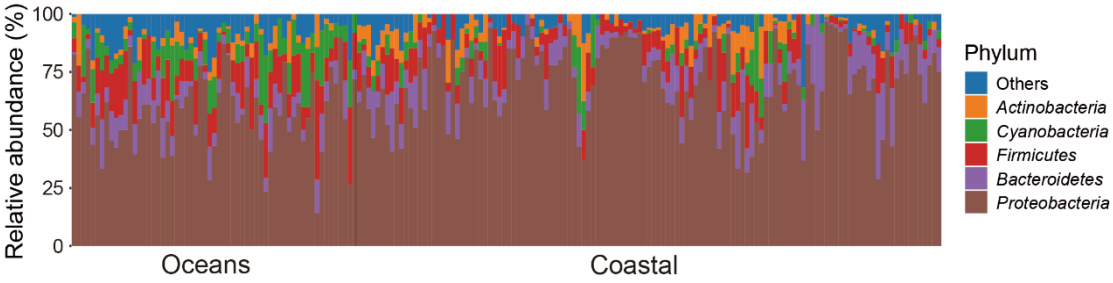

Figure 4-24 Comparison of antibiotic resistance gene (ARG) host composition at the phylum level between oceanic and coastal water samples. The latter had a higher relative abundance of *Proteobacteria*.

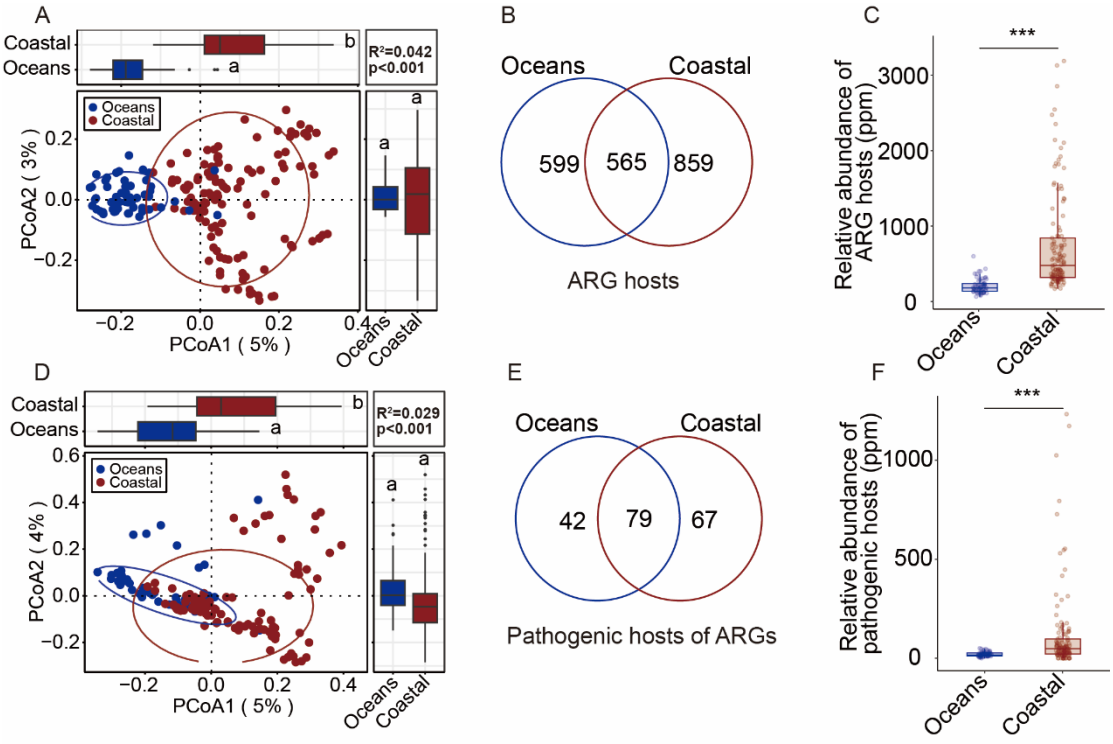

Figure 4-25 Coastal waters harbored significantly different compositions and obviously broader species of resistant bacteria (A and B) and human pathogens (D and E) compared with surface oceans. Their relative abundance was much higher than those in surface oceans (C and F). ARG, antibiotic resistance gene.

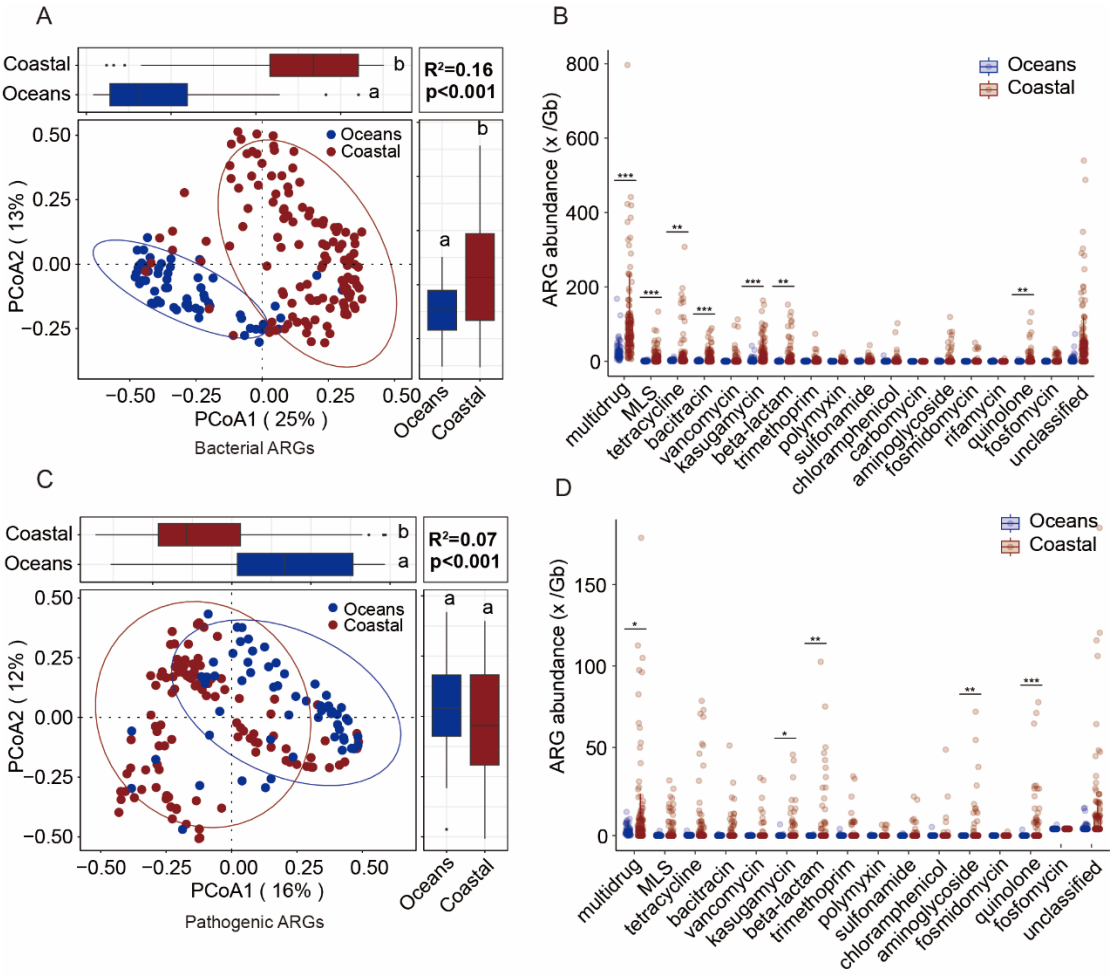

Figure 4-26 Antibiotic resistance gene (ARG) hosts in coastal waters harbored a significantly distinct resistome profile from those in oceanic counterparts (A), enriching the human-associated dominant ARGs (B). There was a similar trend in coastal pathogenic hosts (C and D). MLS, macrolide–lincosamide–streptogramin.

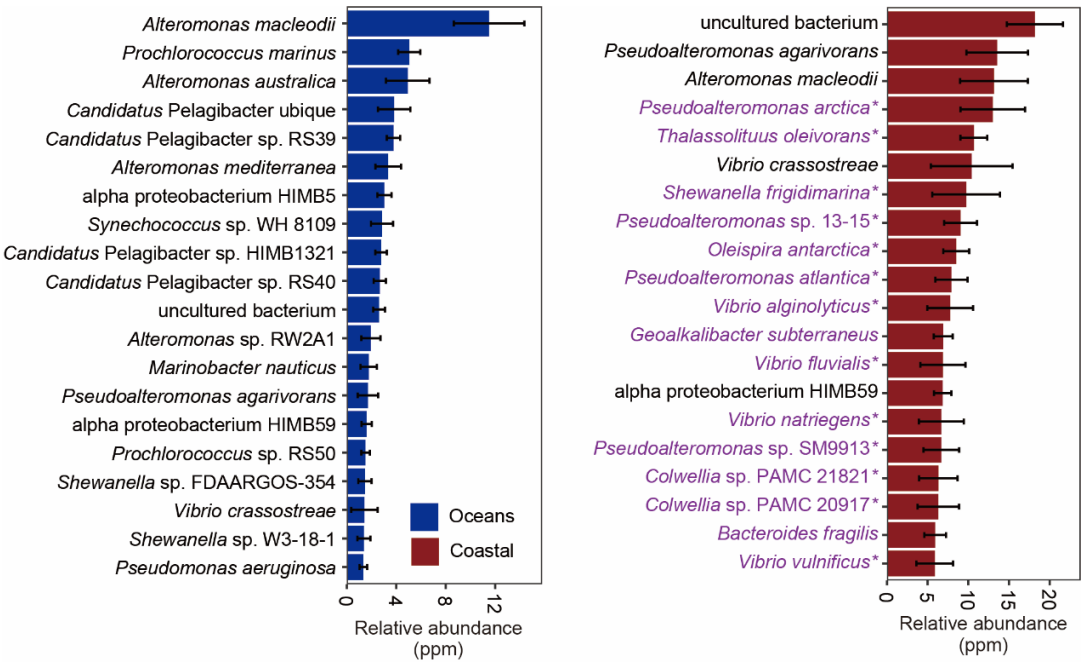

Figure 4-27 Seventy-five percent of the core resistant species (15/20, considering the top 20 species ranked by average relative abundance) presented differences between oceans and coastal waters. Most of the unique core species in coastal waters (marked in purple) belong to the *Gammaproteobacteria* phylum (*).

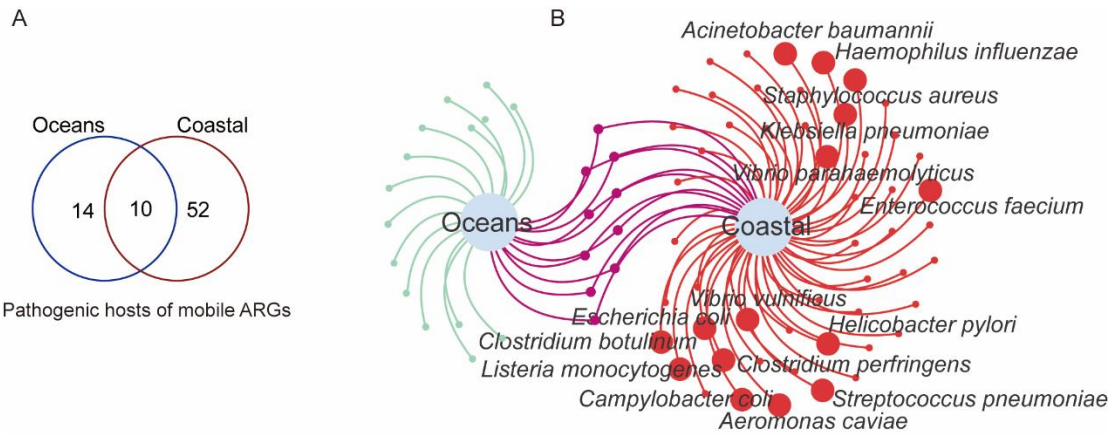

Figure 4-28 Overall, 84% (52/61) of coastal human pathogens carrying mobile antibiotic resistance genes (ARGs) were specific to coastal ecosystems (A), including World Health Organization-designated priority emerging pathogenic species and those regarded as foodborne pathogens (B).

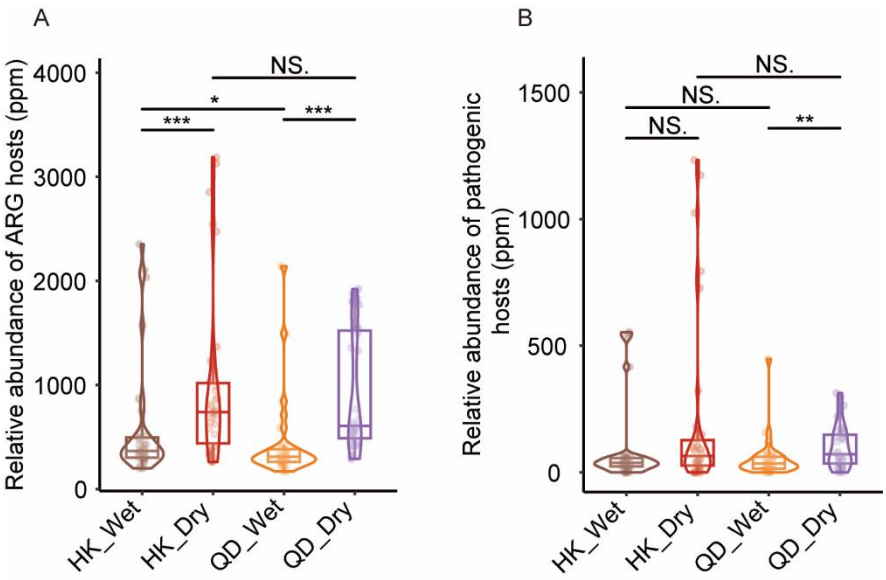

Figure 4-29 Relative abundance of antibiotic resistance gene (ARG) hosts (A) and resistant pathogens (B) increased seasonally from summer to winter in both coastal waters, with no significant regional decline observed in winter.

4.3 Summary

This chapter presents the findings from comparative metagenomic analyses of bacterial and ARG matrices between human-impacted coastal waters and relatively pristine surface oceans to comprehensively evaluate the anthropogenic influences on the natural structure and variations of coastal bacteriomes and resistomes. The principal findings of this investigation are as follows.

1. Human-impacted coastal waters and pristine surface oceans shared the same predominant bacterial phyla. However, *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* were remarkably more abundant in coastal waters compared with oceanic counterparts. Human-impacted coastal waters exhibited obvious shifts in bacterial composition and

differences in 65% of the core species, along with significant biodiversity loss and a notable increase in human pathogens, which involved a significant increase of human-associated bacteria. Human activities have altered the natural structure of coastal bacteriomes, facilitating the proliferation of emerging and foodborne pathogens, such as *E. coli, K. pneumoniae, V. cholerae,* and *V. parahaemolyticus*.

2. Coastal bacterial communities exhibited seasonal and regional variations in their composition at both the phylum and genus levels. Bacterial diversity peaked significantly during winter in both coastal waters, accompanied by a concurrent decline in human pathogen abundance. However, regional variations in human pathogen abundance did not fluctuate similarly to bacterial diversity, with a regional decline in human pathogens observed only during winter. The unexpected discrepancy between the variations in bacterial diversity and pathogen abundance implies that anthropogenic activities have strongly impacted their seasonal and regional variations and diminished the variations in human pathogens.

3. The ARG profile in coastal waters was remarkably different from that in pristine surface oceans, which was associated with the enrichment of human-associated ARGs. Coastal resistomes became more diverse, abundant, and high risk, with stronger mobility and pathogenicity. Its core subtypes exhibited a 35% difference from those in surface oceans. Many high-risk ARGs had the potential to be acquired by human pathogens. There was a clear increase in the relative abundance of plasmid-associated

ARGs from pristine surface oceans to human-impacted coastal waters, with plasmids serving as the primary vectors for HGT. The influence of land-based antibiotic usage and the enhancement in ARG transferability may shape alterations in the natural structure and properties of coastal resistomes.

4. ARG diversity and relative abundance displayed a seasonal increase from summer to winter in both coastal environments. However, regional differences were evident only in winter, characterized by an increase in ARG richness but a reduction in relative abundance from Hong Kong to Qingdao coastal waters. There were similar seasonal and regional trends in structural compositions at the subtype level, showing marked differences between seasonal groups and winter region groups. Such variations in coastal resistomes were derived from the enrichment of human-associated ARGs. Their sharp increases masked the intrinsic seasonal and regional distributions of coastal resistomes. Notably, the elevated distribution of high-risk ARG subtypes during winter underscores an increased exposure risk for the local populations during this season.

5. Coastal ARG hosts demonstrated a broader range and higher relative abundance compared with their oceanic counterparts, including a greater number of emerging and foodborne pathogenic species listed among the priority human pathogens, with 75% of the core bacteria showing differences. The development of these resistant bacteria may be related to the enhanced transmission of human-associated ARGs and the host source inputs.

6. The relative abundance of ARG and pathogenic hosts showed a pronounced seasonal increase, with a slight regional decline in ARG hosts during winter. These seasonal distribution patterns differed notably from those of overall biodiversity, ARGs and human pathogens, pointing to anthropogenic footprints on the natural seasonal and regional variations of coastal resistant bacteria. These influences were likely mediated by the increase in mobility of human-associated ARGs during winter. This seasonal enhancement in mobility may promote the acquisition of resistance by bacteria and human pathogens, contributing to the proliferation of ARG hosts and resistant pathogens during winter and altering their seasonal and regional distributions in coastal waters. These findings suggest that anthropogenic impacts could elevate wintertime exposure risks for coastal populations.

In summary, this investigation underscores the anthropogenic impacts on coastal bacteriomes and resistomes. Coastal waters subject to anthropogenic stress have emerged as ‘hotpots’ for the proliferation of harmful elements. This chapter provides a comprehensive metagenomic study of bacterial and ARG changes in human-impacted coastal waters compared with relatively pristine surface oceans. Nevertheless, there are still knowledge gaps in understanding how anthropogenic activities shape these alterations. To address these gaps, the next chapter elucidates the drivers for these changes, as well as their dominant sources for contamination control management. On the other hand, the increasing prevalence of human pathogens and antimicrobial resistance observed in coastal waters could promote their dissemination into marine

food animals and result in biological contamination of seafood, posing huge challenges for seafood safety. To clarify these processes, Chapter 6 focuses on the mariculture-induced emergence of these biological elements in cultured fishes.

Chater 5 Drivers and health risks of coastal bacteriome and resistome alterations

The comparative metagenomic analysis of bacteriomes and resistomes in human-impacted coastal waters against relatively pristine oceanic environments, as detailed in Chapter 4, uncovered substantial deviations in the characteristics of these microbial components and a marked proliferation of high-risk ARGs and resistant human pathogens. These findings highlight the critical need to unravel the mechanistic drivers behind these microbial shifts and their associated health risks to guide effective contamination mitigation strategies. To address these, we first examined the relationships between bacteriome and resistome characteristics (from diversity to structure) and a collection of environmental and anthropogenic variables: physicochemical parameters, antibiotic concentrations (as selective pressures), crAssphage (indicative of human fecal contamination), and mobile ARGs (reflecting anthropogenically enhanced ARG transmission dynamics). Building on this foundation, we quantified the relative contributions of these factors to identify the principal drivers and traced their origins to pinpoint the dominant anthropogenic activities. Moreover, we assessed potential health risks by establishing phylogenetic linkages between MAG-resolved antibiotic-resistant pathogens from the human-impacted coastal waters and the corresponding clinical strains downloaded from the NCBI RefSeq database, providing insights into the potential for disease outbreaks in coastal populations.

5.1 Drivers of diversity and relative abundance changes

Diversity and relative abundance are critical attributes of coastal bacteriomes and resistomes. To elucidate the factors shaping coastal bacteriomes and resistomes, we examined the relationships of these attributes with the above-mentioned environmental and anthropogenic variables. Correlation analyses revealed that these attributes were strongly influenced by both environmental and anthropogenic factors (Figure 5-1), underscoring their combined role in shaping the status of these microbial systems. The key factors included temperature, antibiotic selection pressure, fecal contamination, and anthropogenically enhanced ARG transfer, all of which exhibited significant correlations with the attributes of coastal bacteriomes and resistomes (Figure 5-1).

Bacterial species richness was inversely correlated with both temperature and antibiotic concentrations (Figure 5-1). Long-term monitoring at the San Pedro Ocean Time-series (SPOT) (Chow et al., 2013) and at a temperate coastal site off Plymouth, UK (Gilbert et al., 2012), revealed recurrent peaks in bacterial diversity during winter over a 6-year period. These studies suggest that lower winter temperatures promote higher bacterial diversity. Consistent with these findings, our results also show elevated bacterial diversity in winter (see Chapter 4) and a negative relationship between temperature and bacterial richness. This suggests that temperature is a key natural factor for the dynamics of bacterial diversity. In contrast, the generally negative correlations between antibiotic concentrations and bacterial richness suggest that increased antibiotic selective pressure contributed to biodiversity loss in coastal waters. This interpretation

aligns with the known inhibitory effects of high antibiotic levels on susceptible bacterial populations (Chakraborty & Bhadury, 2015). However, we also observed a concurrent wintertime increase in both antibiotic concentrations and bacterial richness in both coastal regions (Figures 4-8A and 5-2C), suggesting that the influence of antibiotics during winter may be masked by the combined effects of low temperature and fecal contamination. We found a positive correlation between CrAssphage abundance and bacterial richness (Figure 5-1). It suggests that fecal contamination may introduce new bacterial species into coastal waters, compensating for the loss of bacterial richness induced by antibiotic selection. Meanwhile, the seasonal increase in fecal contamination (Figure 5-2B) also played a compensatory role in richness loss during winter in coastal waters.

Pathogen abundance was positively correlated with both temperature and CrAssphage abundance (Figure 5-1). This finding is consistent with previous studies showing that indigenous marine pathogens thrive under warmer conditions and in regions of elevated temperature (Belkin & Colwell, 2006) and that the influx of human-derived pathogens contributes to the increased abundance of human pathogens in coastal waters (Behera et al., 2023; Blaak et al., 2014; Fresia et al., 2019). These positive correlations, together with the seasonal increase in CrAssphage abundance (Figure 5-2B), support the seasonal decline in pathogen abundance during winter and highlight the anthropogenic contribution to the discrepancy between human pathogens and bacterial diversity patterns, as discussed in Chapter 4. While the influence of temperature represented a

natural process, fecal contamination likely played a key role in amplifying pathogen abundance in coastal waters, interfering with their seasonal and regional variations. Antibiotics may also affect pathogen dynamics. However, their impact appears complex, as both positive and negative relationships with pathogen abundance were observed (Figure 5-1).

The relative abundance of *Proteobacteria*, a dominant bacterial phylum, was negatively correlated with temperature but positively associated with antibiotic concentrations, CrAssphage, and mobile ARGs (Figure 5-1). Consistently, *Proteobacteria* showed seasonal and regional variation patterns similar to those of these anthropogenic factors (Figures 4-6C and 5-2). As temperature represents a natural driver of bacterial dynamics, the expansion and altered variations of *Proteobacteria*, along with the consequent restructuring of core bacterial species in coastal waters, were likely shaped by anthropogenic influences. The influence of antibiotics may be due to their selection favoring the development and proliferation of resistant bacteria in this phylum, supported by an obvious increase in the relative abundance of resistant *Proteobacteria* in coastal waters compared with oceanic systems (Figure 4-24). Fecal contamination also contributed by introducing a large number of bacteria (e.g., *Enterobacteriaceae*) belonging to *Proteobacteria* (Sadowsky & Whitman, 2010). In addition, enhanced ARG transfer may facilitate the acquisition of resistance by *Proteobacteria*, further promoting the proliferation of resistant *Proteobacteria* in coastal water.

Similarly, ARG matrices exhibited strong positive associations and parallel variation patterns with anthropogenic factors (Figures 4-6 A and B, 4-29, 5-1, and 5-2), but significant negative relationships with temperature (Figure 5-1). Previous research has also reported negative correlations between ARG expression and oceanic temperature, suggesting that temperature acts as a natural driver of coastal resistomes. By contrast, numerous studies have linked the emergence of resistomes in human-associated environments to anthropogenic influences (Chen et al., 2017; Karkman et al., 2019; Makkaew et al., 2021). Together, these results indicate that anthropogenic factors were major drivers of coastal resistome proliferation and contributed to their seasonal and regional variability.

In summary, antibiotic selection was the critical driver of biodiversity loss, whereas fecal contamination facilitated the proliferation and variation shifts of human pathogens in coastal waters (Figure 5-3). Together with anthropogenically enhanced ARG transfer, these factors collectively drove the increased prevalence and altered variations of *Proteobacteria* and resistomes in coastal waters (Figure 5-3).

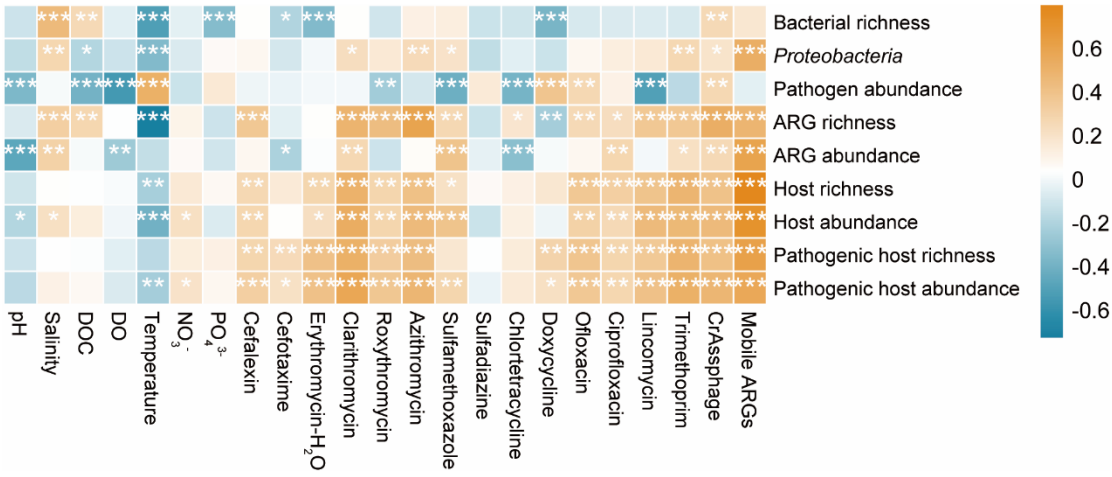

Figure 5-1 Correlations between the attributes of coastal bacteriomes and resistomes and the influencing factors. ARG, antibiotic resistance gene; DO, dissolved oxygen; DOC, dissolved organic carbon.

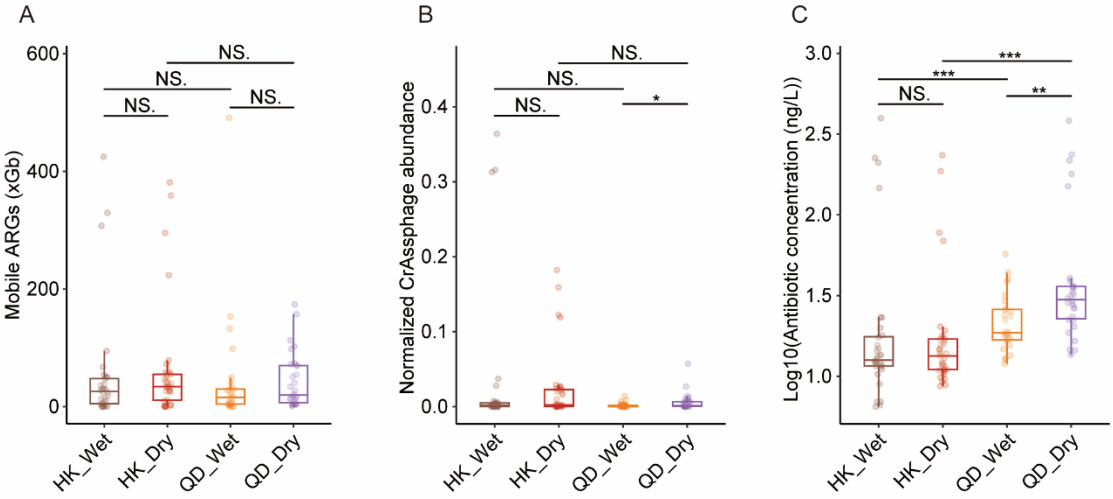

Figure 5-2 Seasonal increases in anthropogenic factors during winter.

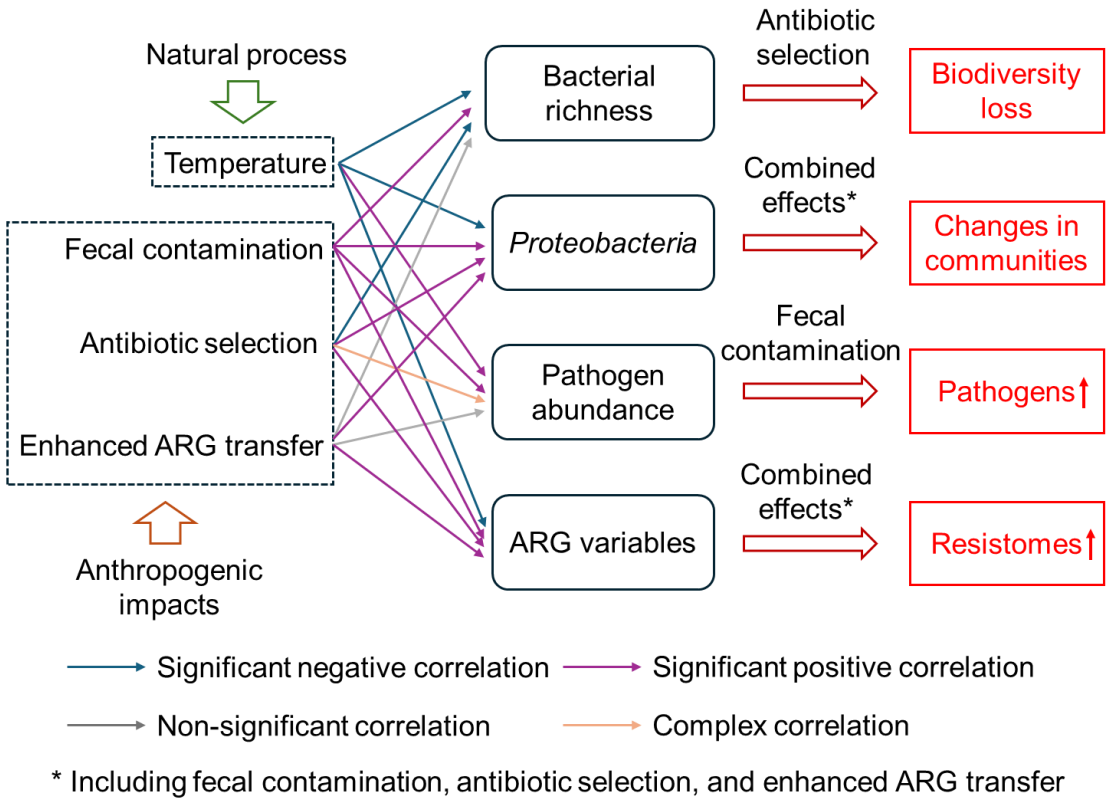

Figure 5-3 Key influencing factors shaping the critical attributes of coastal bacteriomes and resistomes

5.2 Drivers of structural shifts

To elucidate the influence of environmental and anthropogenic factors on the structural shifts in coastal bacteriomes and resistomes, we conducted db-RDA. The analysis revealed that 49.8%, 47.6%, 47.9%, 10.6%, and 6.7% of the structural variation in bacterial communities, human pathogens, ARGs, ARG hosts, and resistant pathogens, respectively, in human-impacted coastal waters could be attributed to these factors (Figures 5-4 and 5-5). Permutation tests under a constrained analysis of principal coordinates (CAPSCALE) confirmed that the structural variations of each bacterial or ARG group were significantly associated with these factors (Figure 5-5), indicating that

the compositional variations of coastal bacteriomes and resistomes were strongly shaped by the combined effects of environmental and anthropogenic drivers. These findings align with the results of earlier correlation analyses, which demonstrated that both environmental and anthropogenic factors jointly influence the diversity and relative abundance of these microbial communities.

Of note, anthropogenic factors were predominantly associated with the winter sample groups, particularly in Hong Kong coastal waters, whereas environmental factors were more closely linked to the summer groups or the winter group of Qingdao coastal waters (Figure 5-4). This suggests that anthropogenic impacts may be responsible for adverse shifts in the attributes of coastal bacteriomes and resistomes during winter, especially in Hong Kong coastal waters. We observed seasonal increases in the levels of anthropogenic factors from summer to winter, with higher concentrations consistently detected in Hong Kong coastal waters, except for the total antibiotic concentration (Figure 5-2). Nevertheless, the effect of antibiotic composition on the structural variations followed a similar seasonal trend to other anthropogenic factors, reaching its peak in Hong Kong coastal waters (Figure 5-6). This implies that antibiotic selection on bacterial communities and ARG profiles depended on both their total concentration and composition. According to these findings, alterations in the structure and variations of coastal bacteriomes and resistomes were primarily due to anthropogenic impacts in winter.

To further assess the relative importance of anthropogenic factors, we employed the 'rdacca.hp' package to disentangle their individual effects. Based on the results, anthropogenically enhanced ARG transfer exerted the strongest influence on the structure of each bacterial and ARG group, followed by fecal contamination and antibiotic selection (Figure 5-5). The comparatively weaker effect of antibiotic selection may be attributed to the dilution of antibiotic concentrations in seawater, which could mitigate their selective pressure.

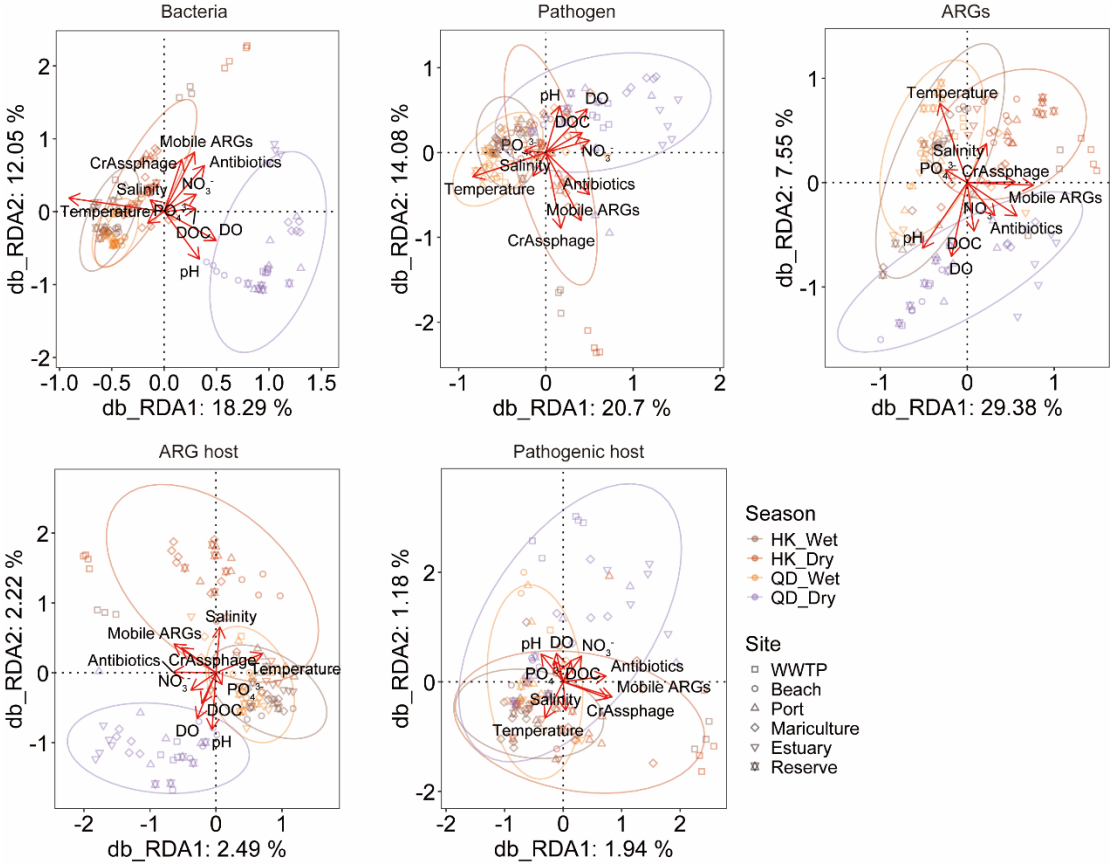

Figure 5-4 Distance-based redundancy analysis (db-RDA) to elucidate the influence of environmental and anthropogenic factors on the structure of coastal bacteriomes and resistomes. ARG, antibiotic resistance gene; DO, dissolved oxygen; DOC, dissolved organic carbon.

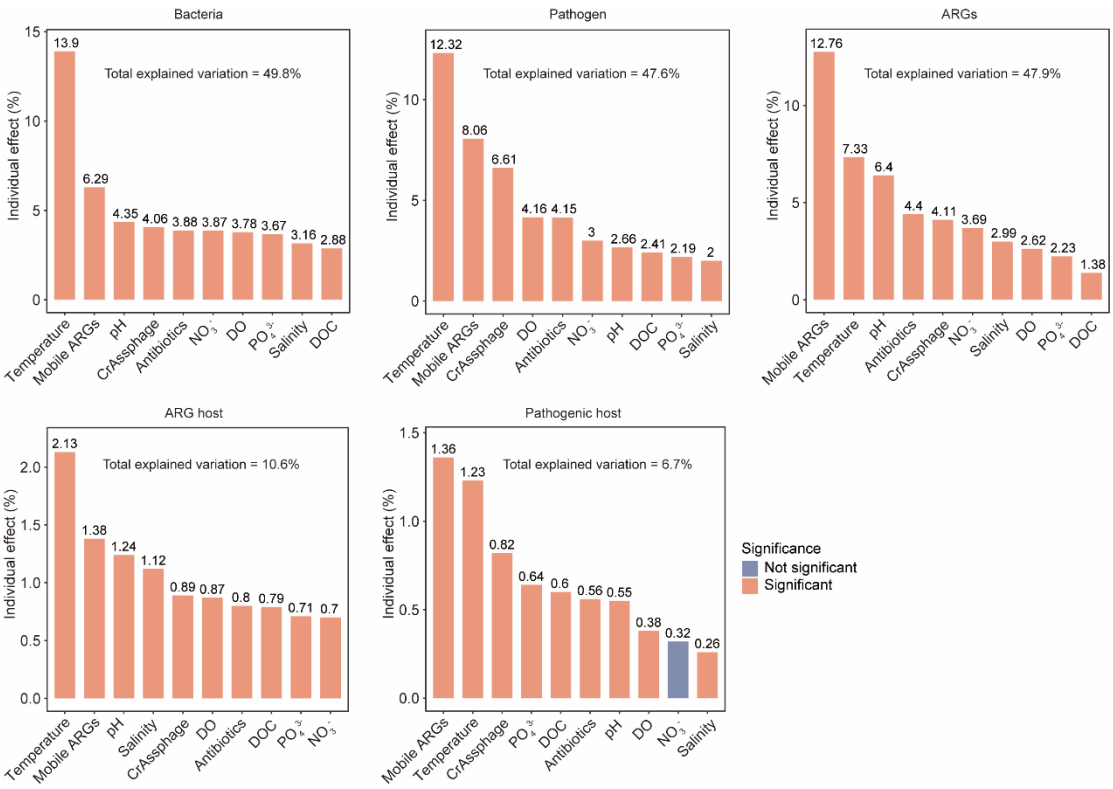

Figure 5-5 Individual effect of different influencing factors on the structural variations revealed by the 'rdacca.hp' package. ARG, antibiotic resistance gene; DO, dissolved oxygen; DOC, dissolved organic carbon.

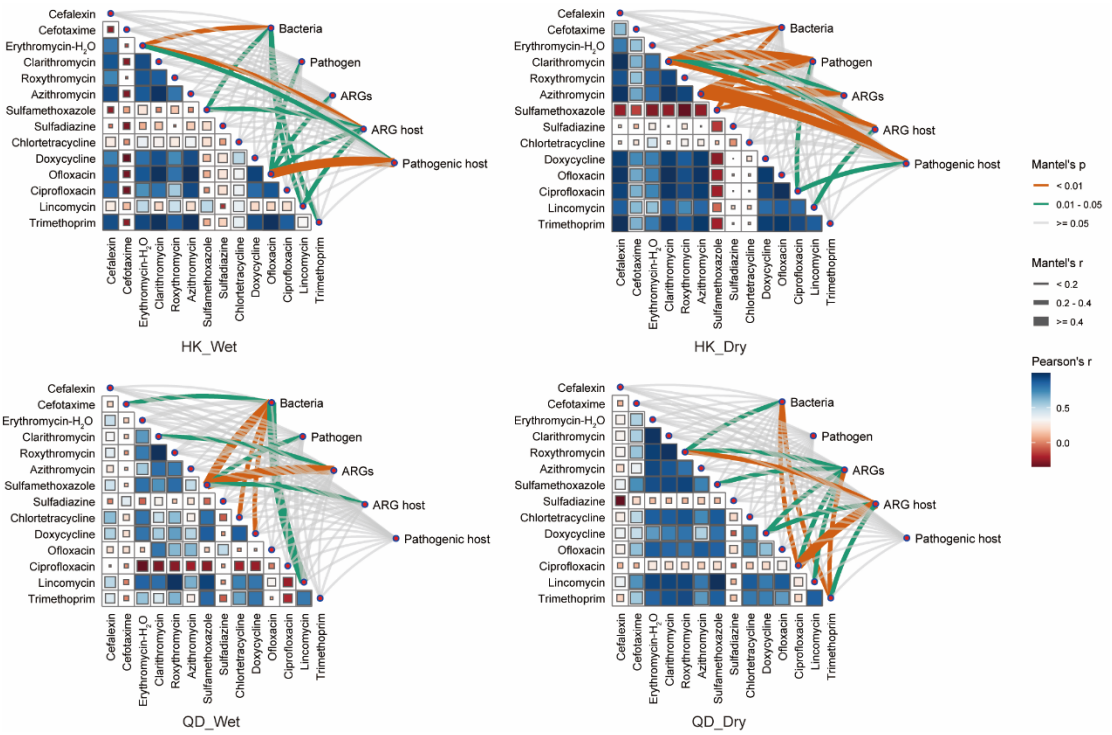

Figure 5-6 Partial Mantel tests demonstrated that the effects of antibiotic composition on the structural variations exhibited a seasonal increase in winter and a highest level in Hong Kong coastal waters. ARG, antibiotic resistance gene.

5.3 Key origins of the anthropogenic drivers

To pinpoint the primary origins of anthropogenic factors for contamination management, we analyzed the concentrations of these factors across coastal sites in two cities during the winter season. As illustrated in the Figure 5-7, there were elevated levels of these factors in the receiving water of WWTPs in Hong Kong coastal regions, whereas we generally observed higher concentrations in the estuarine site along the Qingdao coast. Based on these patterns, WWTP discharge and contaminated riverine runoff represented the dominant origins of anthropogenic factors in Hong Kong and Qingdao, respectively. This observation aligns with recent studies reporting elevated

antibiotic concentrations, strong correlations between MGEs and ARGs, and significant fecal bacteria contamination in these areas (Fresia et al., 2019; Zhou et al., 2022). Such origins have been identified as major anthropogenic stressors of alterations in coastal bacteriomes and resistomes (Zheng et al., 2021). A subsequent comparative analysis of regional concentrations indicated that, except for the total antibiotic level, these factors were consistently higher in the receiving water of Hong Kong WWTPs compared with the Qingdao estuarine site. This disparity was likely attributable to the lower treatment efficiency of Hong Kong WWTPs, which utilize a chemically enhanced primary treatment process, thereby releasing substantial loads of human-associated bacteria and genetic elements into the receiving water. This finding corroborates the results of db-RDA analysis, providing further explanation for the more pronounced alterations in the characteristics of coastal bacteriomes and resistomes in Hong Kong coastal waters during winter. Notably, the concentrations of discriminative bacterial and ARG elements generally mirrored those of the anthropogenic factors in their respective origins, offering additional evidence to support these conclusions (Figure 5-8).

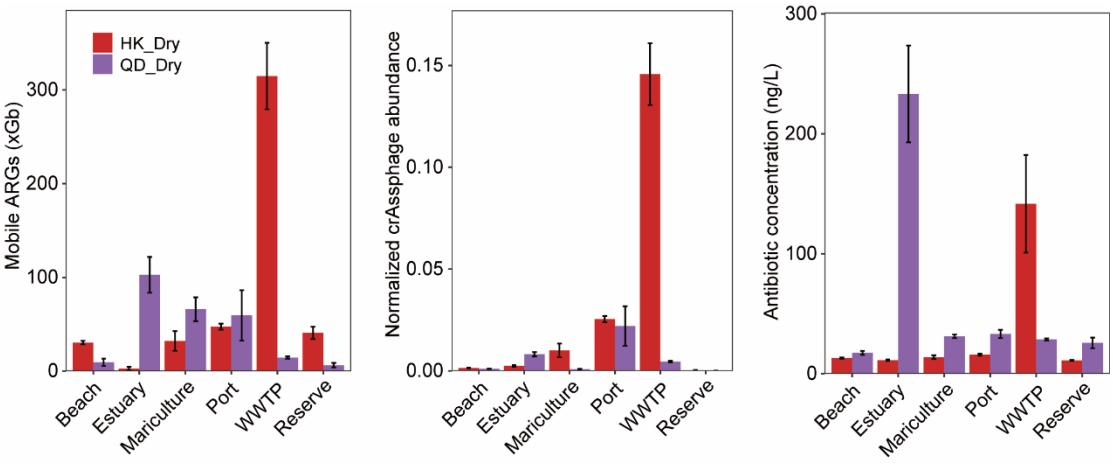

Figure 5-7 Concentrations of anthropogenic factors across various impacted sites in two coastal regions during winter. ARG, antibiotic resistance gene; WWTP, wastewater treatment plant.

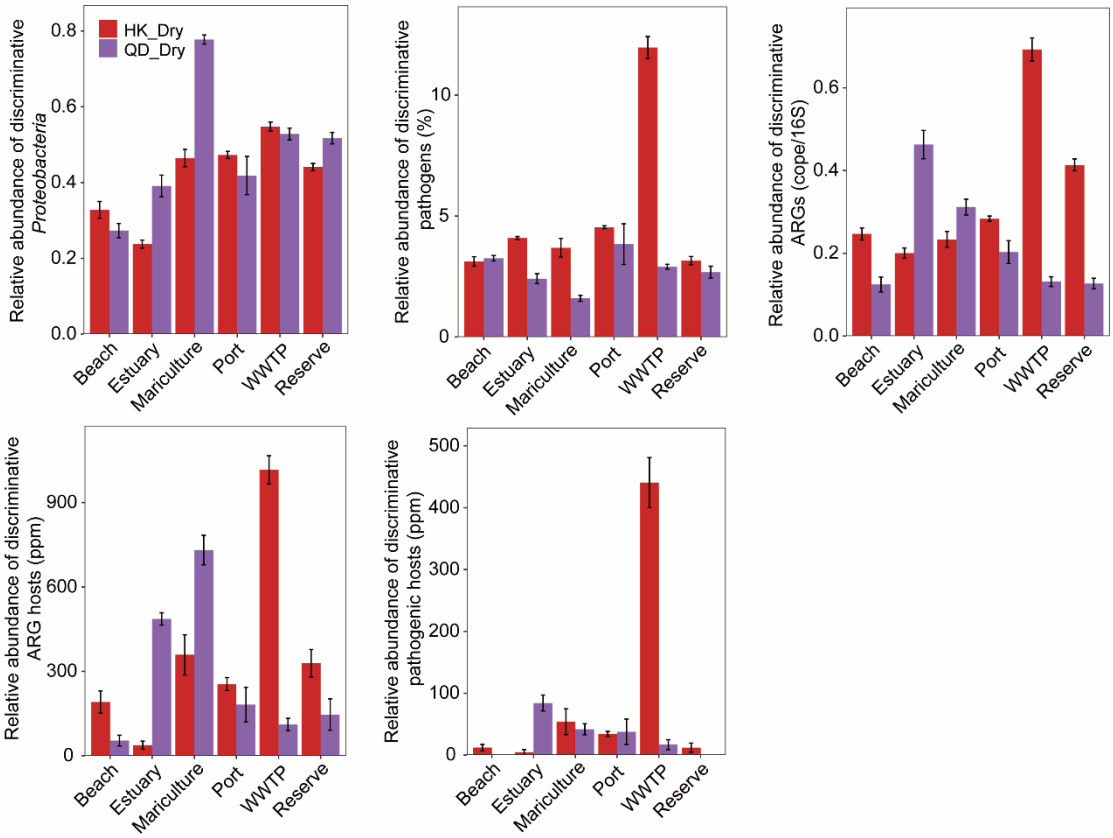

Figure 5-8 The relative abundance of discriminative elements in *Proteobacteria*, human pathogens, resistomes, antibiotic resistance gene (ARG) hosts, and pathogenic hosts

across various impacted sites in two coastal regions during winter. WWTP, wastewater treatment plant.

5.4 Anthropogenically enhanced health risks of coastal bacteriomes and resistomes

Considering the alterations in the natural structure of bacteriomes and resistomes and the proliferation of high-risk ARGs and resistant pathogens in coastal environments, further research is warranted to elucidate their health risks. In this context, we conducted phylogenetic analysis between MAG-resolved resistant pathogens from human-impacted coastal waters and the clinical strains previously implicated in diseases outbreaks retrieved from the NBCI RefSeq database.

5.4.1 The presence of genome-resolved *E. coli* and *A. caviae* with multidrug resistance

Metagenomic binning yielded 1,928 and 1,611 high-quality MAGs from coastal and oceanic samples, respectively. Of these MAGs, we identified four coastal genomes as pathogens: *A. caviae*, *Bacteroides uniformis*, *E. coli*, and *Vibrio fluvialis*. These pathogens, except for *B. uniformis*, carried multiple ARGs, including those conferring resistance to key human and animal medicines (Figure 5-9). The presence of these superbugs underscores the anthropogenic contributions to antimicrobial resistance contamination and the proliferation of resistant human pathogens in coastal ecosystems. Source tracking reveals that these superbugs originated from the winter receiving

waters of Hong Kong WWTPs. This result agrees with the above finding and the recent documentation of antibiotic-resistant pathogens in both WWTPs and their receiving waters (Blaak et al., 2014; Cacace et al., 2019; Kvesić et al., 2022; Rodriguez-Mozaz et al., 2015). The combination of extremely high bacterial loads and subtherapeutic concentrations of chemical agents may create high levels of genetic elements and selective pressures for the evolution of commensal bacteria into clinically relevant resistant strains in both WWTPs and their receiving environments (Rizzo et al., 2013). The release of these pathogens, whether from WWTPs or through *in situ* generation, may ultimately trigger the prevalence of harmful strains in coastal ambient settings.

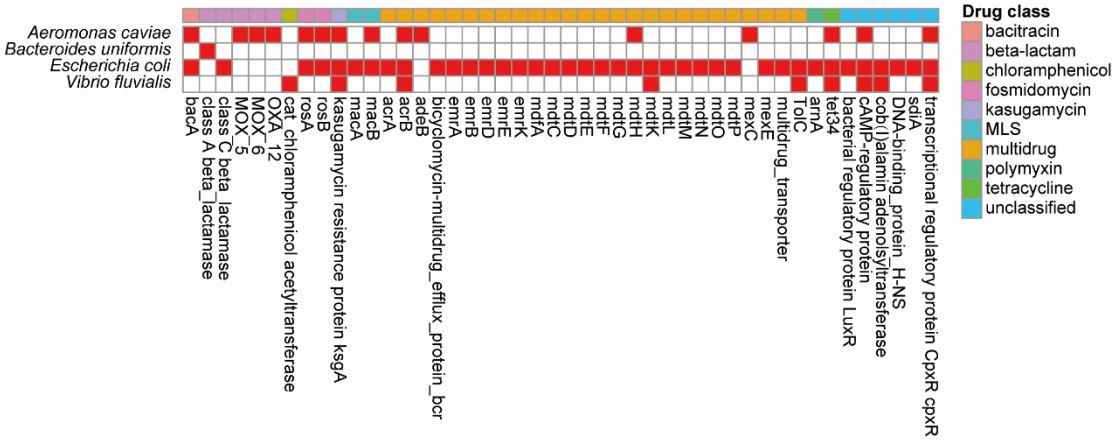

Figure 5-9 Multiple antibiotic resistance genes (ARGs) were detected in genome-resolved human pathogens from coastal waters. MLS, macrolide – lincosamide – streptogramin.

5.4.2 Phylogenetic evidence of infection risks in coastal communities

To investigate phylogenetic relationships between the MAG-resolved resistant pathogens from human-impacted coastal waters and the clinical strains previously

implicated in disease outbreaks, we retrieved all complete genomes of the corresponding species from the NCBI RefSeq database, which includes the clinical isolates. In total, 2,490 *E. coli*, 36 *A. caviae*, 10 *V. fluvialis*, and 7 *B. uniformis* genomes were downloaded. Based on phylogenetic analysis, only MAG-resolved multidrug-resistant *E. coli* and *A. caviae* from human-impacted coastal waters exhibited strong phylogenetic proximity to the resistant clinical strains of extended spectrum beta-lactamase (ESBL)-producing *E. coli* (Hassan et al., 2011) and *A. caviae FDAARGOS_72* (Klemm et al., 2024), respectively (Figure 5-10). ESBL-producing *E. coli* has been recognized globally as an emerging pathogen responsible for a range of community-acquired infections, such as urinary tract infections (UTIs) and bloodstream infections, often with limited therapeutic options (Bezabih et al., 2021; Hassan et al., 2011; Quan et al., 2017). Similarly, resistant *A. caviae FDAARGOS_72* is an enteric pathogen of clinical importance linked to diarrhea outbreaks in young children (Aguilera-Arreola et al., 2007; Beatson Scott et al., 2011; Klemm et al., 2024). Our findings indicate that the presence of these superbugs in coastal waters could lead to serious outbreaks of community-based infections via marine transmission routes, for example, recreational activities. This phenomenon underscores the urgent need for robust surveillance of pathogen dynamics in coastal environments and their transmission pathways back to coastal populations under the One Health framework. Marine pathways become crucial in disseminating anthropogenically induced resistant pathogens into coastal populations, a fact that has been overlooked in previous studies, contributing to the global health burden of these harmful bacteria. Greater attention

must be given toward this issue in future antimicrobial resistance management strategies.

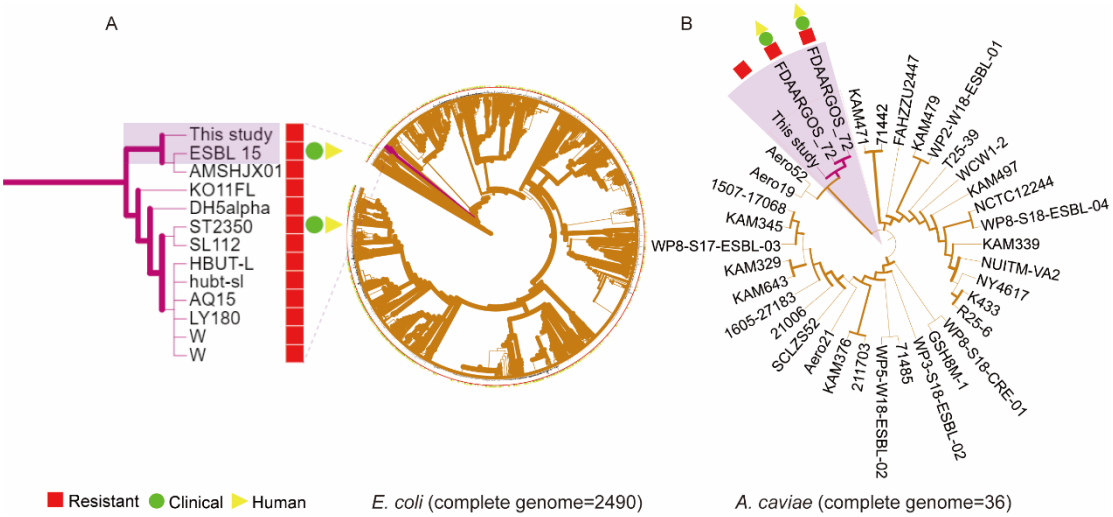

Figure 5-10 Multidrug-resistant *Escherichia coli* and *Aeromonas caviae* detected in coastal waters exhibited a close phylogenetic relationship with the clinical resistant strains of extended spectrum beta-lactamase (ESBL)-producing *E. coli* (A) and *A. caviae* FDAARGOS_72 (B) causing community-based infections, respectively.

5.5 Summary

We elucidated the drivers and health risks associated with microbial alterations in coastal ecosystems. The key findings of this chapter are summarized as follows.

1. Temperature, antibiotic selection, fecal contamination, and anthropogenically enhanced ARG transfer were the crucial factors influencing the diversity and relative abundance of coastal bacteriomes and resistomes. Among these, antibiotic selection was the critical driver of biodiversity loss, while fecal contamination facilitated the

proliferation and variation shifts of human pathogens in coastal waters. Antibiotic selection, fecal contamination, and anthropogenically enhanced ARG transfer jointly drove the increased prevalence of *Proteobacteria* and resistomes in coastal waters.

2. The composition of coastal bacteriomes and resistomes was significantly influenced by the combined effects of environmental and anthropogenic factors. The elevated levels of anthropogenic factors during winter, particularly in Hong Kong coastal waters, were likely responsible for the proliferation of human-associated bacteria and ARGs, thereby driving the adverse shifts in the properties and structure of these microbial components. Anthropogenically enhanced ARG transfer emerged as the predominant driver, followed by source dissemination and antibiotic selection. WWTP discharge and contaminated riverine runoff were identified as the primary origins of these factors in Hong Kong and Qingdao, respectively.

3. MAG-resolved *E. coli and A. caviae* harbored multidrug ARGs and showed close phylogenetic relationships with the resistant clinical strains of ESBL-producing *E. coli and A. caviae* FDAARGOS_72*,* previously implicated in community-based UTIs and diarrhea, indicating their linkages to the community-based infections in coastal regions. The detection of these pathogens in coastal waters highlights the elevated health risk of coastal microbial alterations, which was associated with the exposure of these superbugs to coastal communities via recreational activities.

In summary, our findings underscore the critical role of anthropogenic factors during winter, particularly in Hong Kong coastal waters, in reshaping coastal microbial communities and resistance profiles. We identified WWTP discharge and contaminated riverine runoff as the primary anthropogenic sources of these factors. MAG-resolved resistant pathogens further highlight the risk of community-acquired infections in coastal regions through waterborne and foodborne transmission. To better understand how human-derived contamination spreads from the environment to marine life and potentially disseminates throughout the food chain into human, with implications for the health of coastal populations, we planned to sample wild animals (e.g., fish) in the most impacted environments. However, these efforts were hindered by the fishing ban. Meanwhile, we observed the proliferation of human pathogens, antimicrobial resistance, and host bacteria in mariculture zones in both coastal regions (see Figure 5-8). Given that seafood from mariculture represents a vital source of protein and nutrition for the global diet, this raises a critical question: could mariculture-induced environmental changes trigger the dissemination of these elements from the environment into cultured seafood, thereby leading to seafood contamination and exposure risks to humans via seafood processing and consumption? To answer this question, we selected a representative mariculture farm located in the Hong Kong fish culture zone for further study. The details are provided in Chapter 6.

Chater 6 Mariculture-induced emergence and health implications of human pathogens and antibiotic resistomes in cultured fishes

The elevated levels of human pathogens, resistomes, and ARG hosts in mariculture environments, as illustrated in Chapter 5 by metagenomic analysis, suggest the potential for their dissemination and accumulation in cultured animals, raising concerns over seafood safety and foodborne transmission. This necessitates a thorough assessment of the emergence, underlying formation mechanisms, and health risks of these biological elements in cultured seafood related to mariculture-induced environmental alterations. Therefore, we conducted a metagenomic insight into the enrichment and source dynamics of these elements in various farmed fish species with different feeding behaviors. These samples were collected from a representative mariculture farm under the Accredited Fish Farm Scheme in the Sai Kung coastal waters of Hong Kong during summer and winter. Furthermore, we investigated ARG transmission within the mariculture system to elucidate its role in the accumulation of ARGs and the restructuring of ARG profiles in resistant pathogens. Based on these findings, we evaluated the influences of environmental changes and other mariculture-related factors on the emergence of these elements in farmed fishes. Finally, we conducted culture-based monitoring of resistant pathogens in farmed fishes and evaluated their phylogenetic relationships with the clinical strains previously implicated in disease outbreaks to assess potential health implications for coastal communities.

6.1 Enrichment of human pathogens in cultured fishes

Using the read-based metagenomic analysis, we identified a total of 359 pathogenic species in the collected samples. The composition of these pathogens in fish skins and guts differed significantly from those in the environmental and feed samples (Figure 6-1A and B, PERMANOVA, $p_{Skin} < 0.001$, $p_{Gut} < 0.001$), with a marked increase in their relative abundance (Figure 6-2A). This result highlights the pronounced enrichment of human pathogens in cultured fishes, which involved a sharp rise in emerging and foodborne pathogens, such as *S. aureus, K. pneumoniae, and E. coli* (Figure 6-2B). Together, these pathogens accounted for over 60% and 43% of the total pathogen load in fish skins and guts, respectively (Figure 6-2C).

PCoA revealed that the composition of human pathogens in fish skins and guts varied according to feeding behavior (Figure 6-3A and B, PERMANOVA, $p_{Skin} < 0.001$, $p_{Gut} < 0.001$). However, there were no significant differences in pathogen composition between omnivorous and herbivorous fish skins (Pairwise.adonis, $p_{TBvsSC} = 0.287$), nor between carnivorous and herbivorous fish guts (Pairwise.adonis, $p_{ECvsSC} = 0.187$, $p_{EFLvsSC} = 0.119$). Similarly, there were no marked distinctions in pathogen composition between carnivorous species in the skins (Pairwise.adonis, $p_{ECvsEFL} = 0.410$) or guts (Pairwise.adonis, $p_{ECvsEFL} = 0.100$). In contrast to the pronounced interspecies differences in pathogen composition other researchers have observed in wild marine fishes across different feeding behaviors (Egerton et al., 2018; Korry & Belenky, 2023) , the patterns in cultured fishes were considerably attenuated. Pathogen abundance was

generally higher in both the skins and guts of omnivorous and herbivorous species compared with carnivorous species (Figure 6-4A and B), with seasonal variations observed in omnivorous fish guts and herbivorous fish skins and guts (Figure 6-4C and D). Further analysis of the dominant pathogenic species showed that *S. aureus and K. pneumoniae* were more abundant in omnivorous and herbivorous fish skins and in omnivorous fish guts (Figure 6-5). The seasonal variations in the relative abundance of these pathogens exhibited similar trends and correlated with the total pathogen abundance (Figure 6-6). The enrichment of these dominant pathogens likely contributed to the elevated levels of human pathogens in these tissues (Figure 6-4A and B).

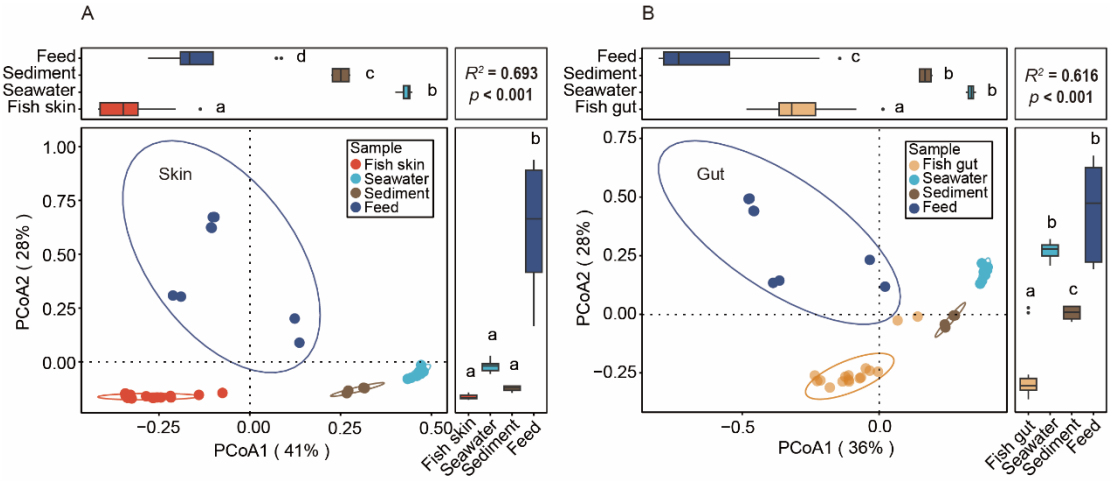

Figure 6-1 The composition of human pathogens in fish skins (A) and guts (B) differed significantly from those in the environmental and feed samples.

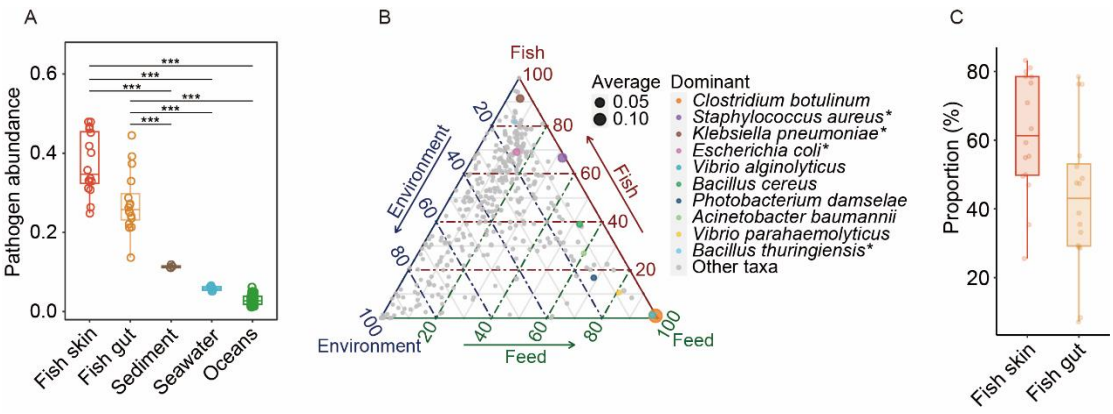

Figure 6-2 Pathogen abundance was significantly elevated in fish skins and guts compared with environmental samples and healthy marine systems (A). The accumulation of human pathogens involved a sharp increase in emerging and foodborne pathogens, such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli* (B). *Staphylococcus aureus*, *K. pneumonias*, and *E. coli* accounted for a very large proportion of the total fish pathogen abundance in skins and guts (C).

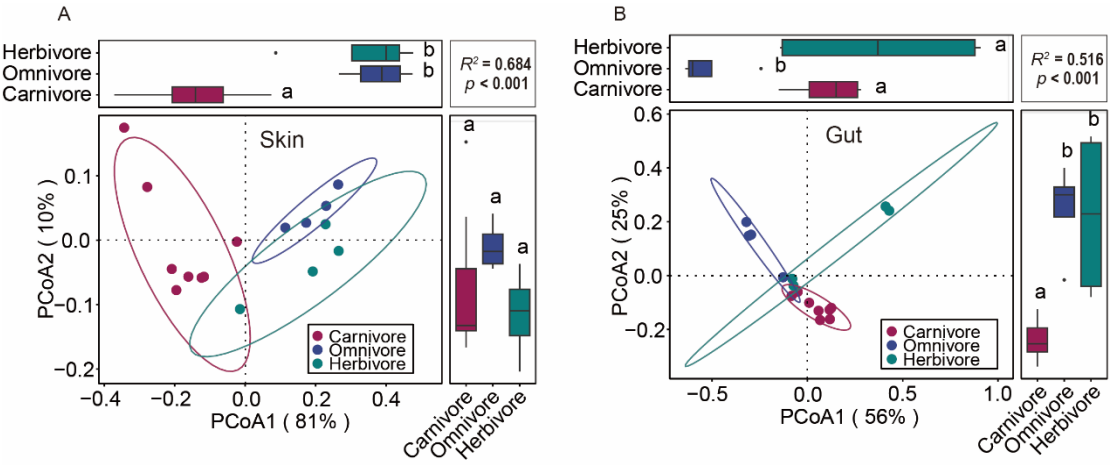

Figure 6-3 The structural compositions of human pathogens at the species level in skins (A) and guts (B) varied according to the feeding behavior.

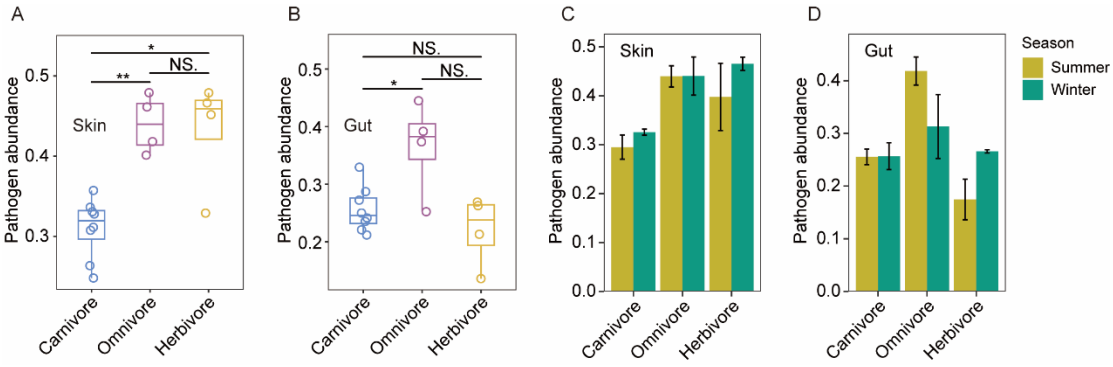

Figure 6-4 Pathogen abundance was generally higher in both the skins (A) and guts (B) of omnivorous and herbivorous fish compared with carnivorous species, with seasonal variations observed in omnivorous fish guts and herbivorous fish skins and guts (C and D).

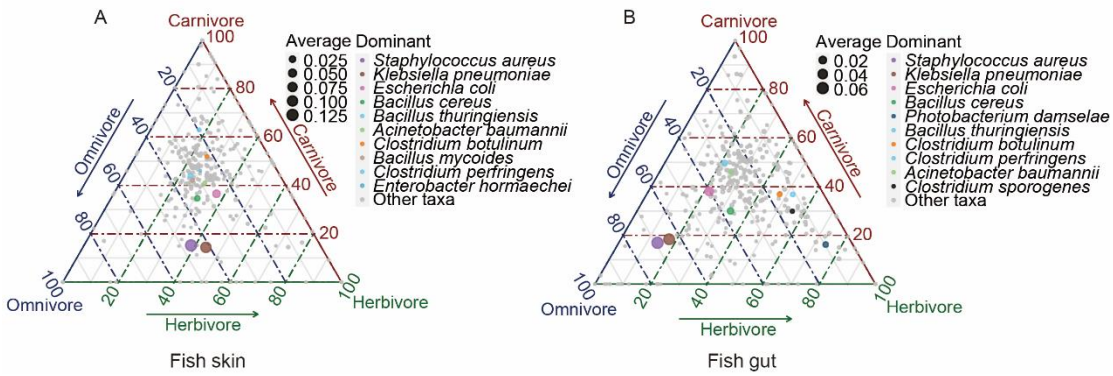

Figure 6-5 *Staphylococcus aureus* and *Klebsiella pneumoniae* were more abundant in omnivorous and herbivorous fish skins (A) and in omnivorous fish guts (B).

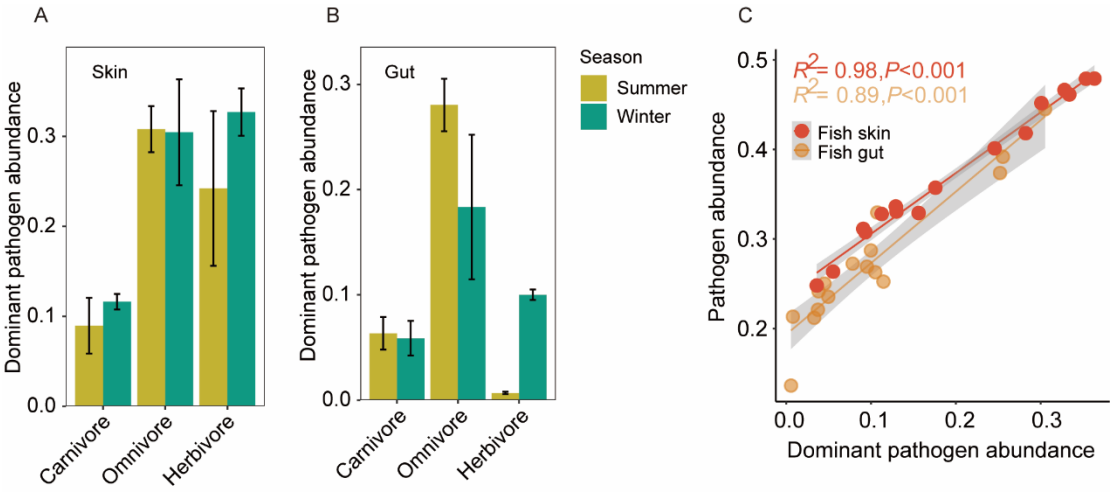

Figure 6-6 Seasonal variations in the relative abundance of *Staphylococcus aureus* and *Klebsiella pneumoniae* exhibited similar trends (A and B) and correlated with the total pathogen abundance (C).

6.2 Accumulation of typical ARGs in cultured fishes

ARGs in the collected samples were characterized and quantified using read-based metagenomics. The resistome profiles at the subtype level in fish skins and guts were significantly distinct from those found in environmental and feed samples (Figure 6-7A and B, PERMANOVA, $p_{Skin} < 0.001$, $p_{Gut} < 0.001$). We identified a total of 21 ARG types in the fish samples, with the predominant resistance against novobiocin, vancomycin, tetracycline, multiple drugs, mupirocin, trimethoprim, chloramphenicol, MLS antibiotics, and bacitracin (Figure 6-8). Environmental and feed samples exhibited a broader range of ARG types, with a total of 29 ARGs (Figure 6-8). Despite this higher diversity, the dominant types in the environments and feeds closely mirrored those in the fishes, except for polymyxin and beta-lactam resistance. These results suggest that the mariculture system and farmed fishes harbored a broad-spectrum

resistome profile.

Although the relative abundance of ARGs was lower in fish skins and guts compared with environmental and healthy oceanic systems (Figure 6-9A), typical ARGs were enriched in fish samples (Figure 6-9B). The dominant ones included *vanHF, vanUG, vat(B), lsa(B), LlmA* 23S ribosomal RNA methyltransferase, *vat(E), mel, DfrA43_TMP, catD*, and *C. coli* chloramphenicol acetyltransferase, which confer resistance to vancomycin, MLS antibiotics, trimethoprim, and chloramphenicol (Figure 6-9B). These genes constituted over 20% of the resistome abundance in fish skins and guts (Figure 6-9C).

Carnivorous, omnivorous, and herbivorous species exhibited significantly distinct profiles of ARG subtypes in both skins and guts (Figure 6-10A and B, PERMANOVA, $p_{Skin} < 0.001$, $p_{Gut} < 0.001$). However, the resistome composition in carnivorous species showed significant similarity between their skins (Pairwise.adonis, $p_{ECvsEFL} = 0.977$) and guts (Pairwise.adonis, $p_{ECvsEFL} = 0.875$), which contrasts with the interspecies variations observed in wild marine piscivorous fishes from coastal New England (Korry & Belenky, 2023). This result suggests that interspecies differences in the resistome composition were reduced among farmed fishes relative to wildlife. Analysis of the relative abundance demonstrated an increasing trend in ARG abundance from carnivorous to herbivorous fish skins and guts (Figure 6-11A and B). Herbivorous fish skins and guts exhibited a marked seasonal decline in ARG abundance during winter;

we did not observe such a pattern in the other fish species (Figure 6-11C and D). The enriched ARGs were more prevalent in omnivorous and herbivorous fish skins and guts (Figure 6-12A and B), displaying relative abundance trends that paralleled those of total ARGs across fish species and seasons (Figure 6-13A and B). The dynamics of these ARGs correlated closely with the overall trends in total ARG abundance (Figure 6-13C). These observations reflect that the increase in these ARGs has significantly influenced the fish resistome profiles.

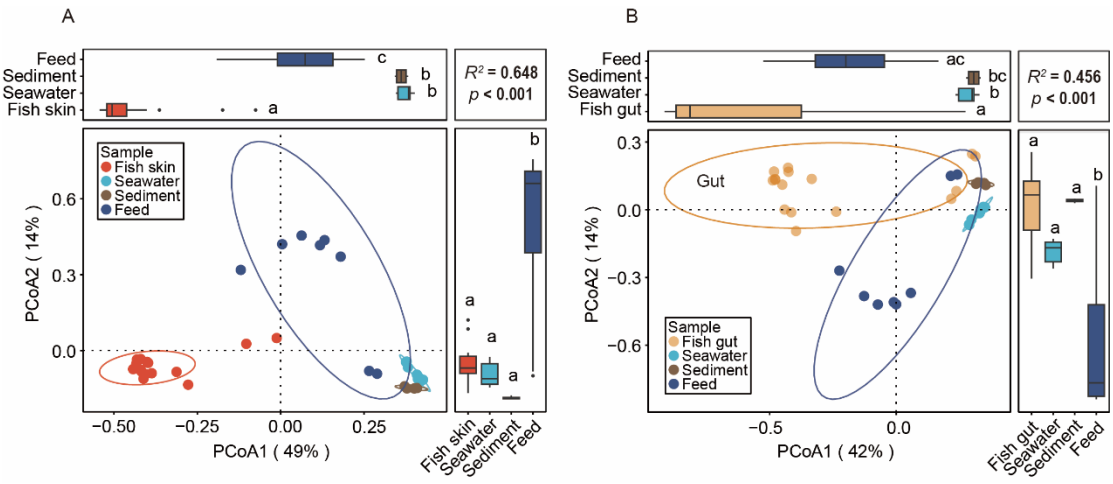

Figure 6-7 The resistome profiles at the subtype level in fish skins (A) and guts (B) were significantly distinct from those found in the environmental and feed samples.

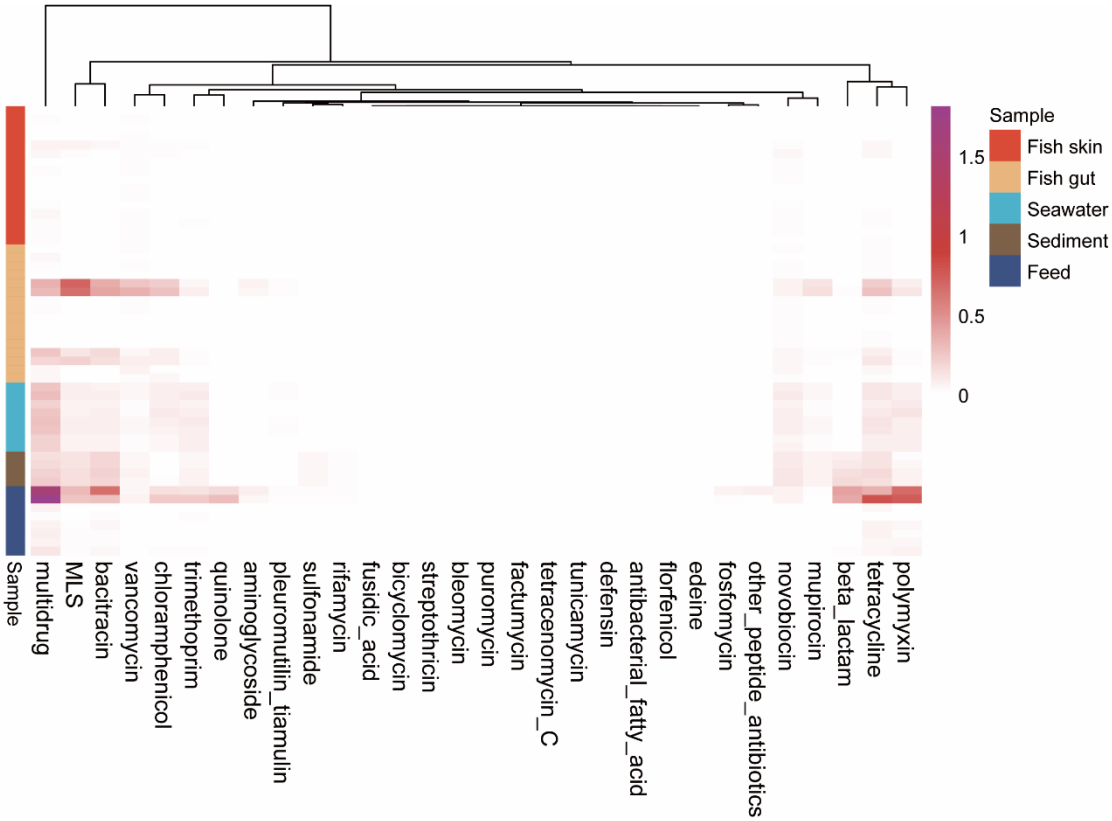

Figure 6-8 A broad spectrum of antibiotic resistance gene (ARG) types was prevalent in the mariculture system and farmed fishes. MLS, macrolide – lincosamide – streptogramin.

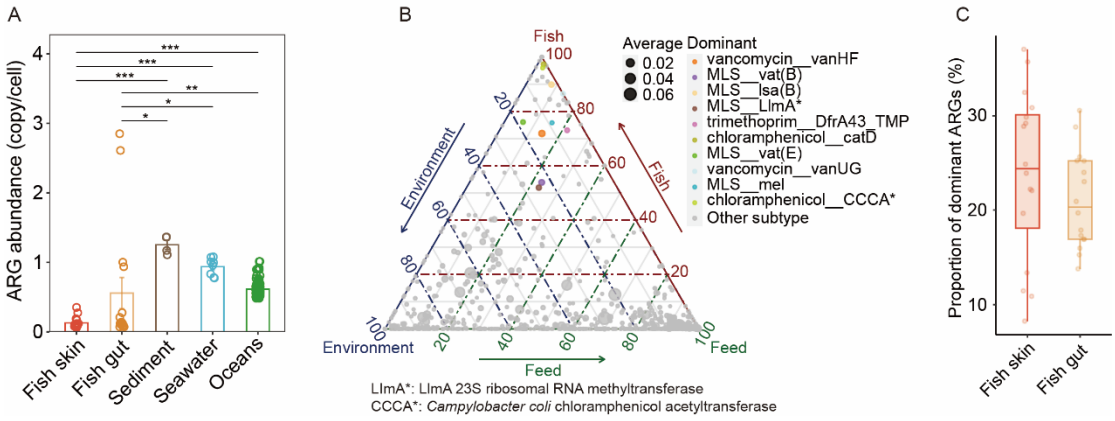

Figure 6-9 The relative abundance of antibiotic resistance genes (ARGs) was lower in fish skins and guts compared with the environmental and healthy oceanic systems (A). Specific ARGs were enriched in fish samples (B). The dominant ones constituted over

20% of the resistome abundance in fish skins and guts (C). MLS, macrolide–lincosamide–streptogramin.

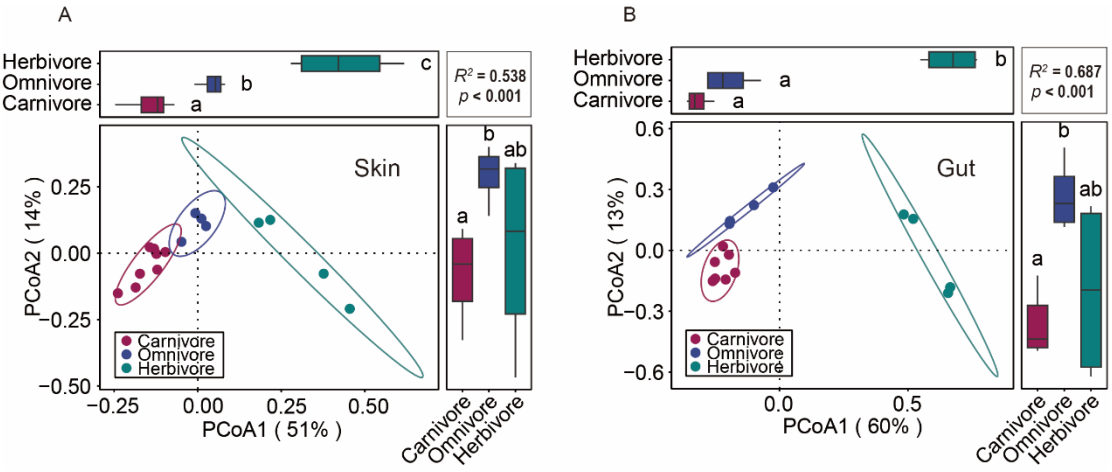

Figure 6-10 Carnivorous, omnivorous, and herbivorous fish exhibited significantly different antibiotic resistance gene (ARG) subtype profiles in both skins (A) and guts (B).

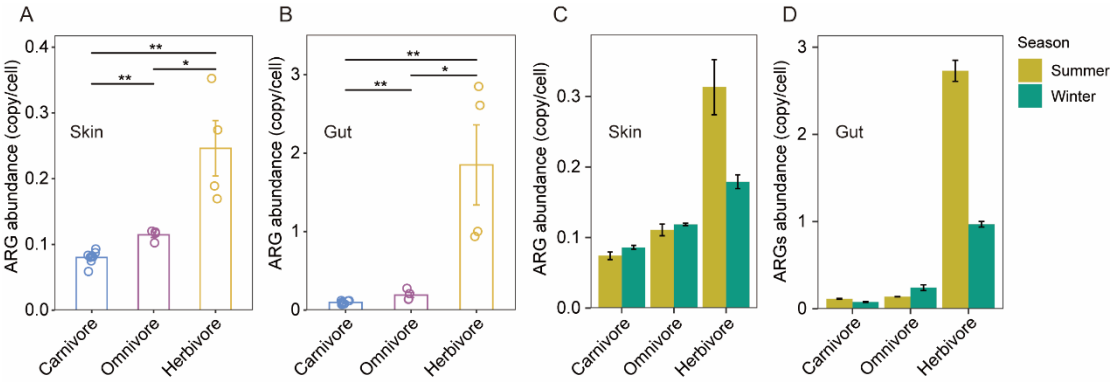

Figure 6-11 There was an increasing trend in antibiotic resistance gene (ARG) levels from carnivorous to herbivorous fish skins (A) and guts (B). Herbivorous fish skins (C) and guts (D) exhibited a marked seasonal decline in ARG abundance during winter.

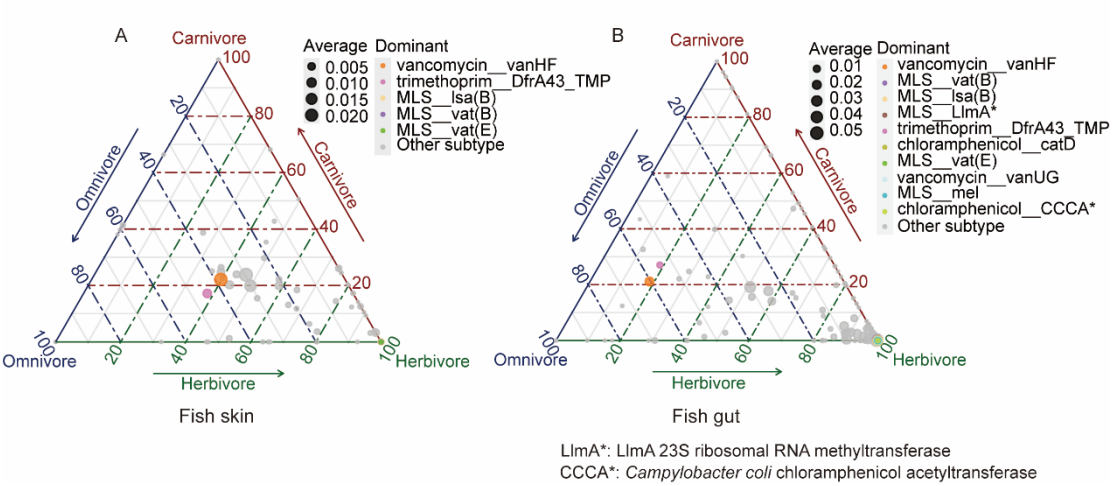

Figure 6-12 The enriched antibiotic resistance genes (ARGs) were more prevalent in omnivorous and herbivorous fish skins (A) and guts (B). MLS, macrolide – lincosamide–streptogramin.

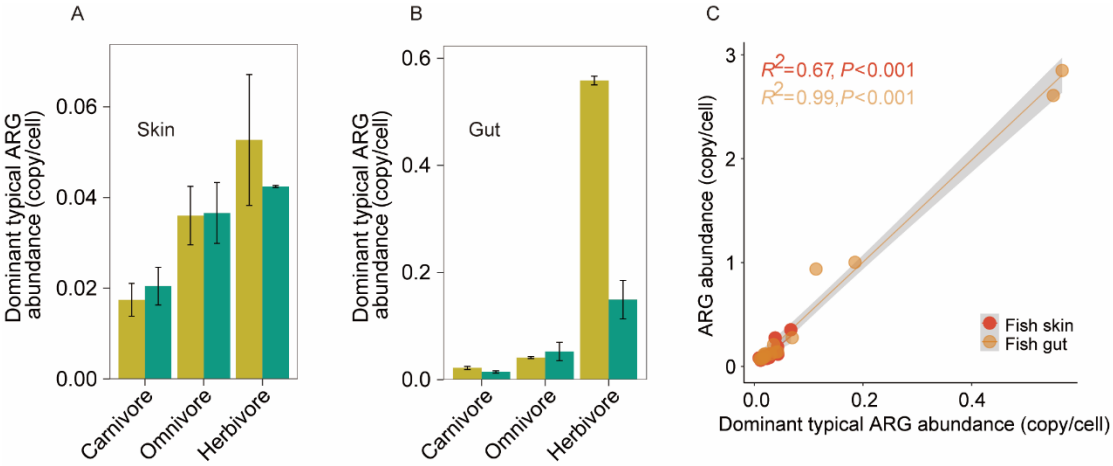

Figure 6-13 The enriched antibiotic resistance genes (ARGs) in fish skins (A) and guts (B) displayed relative abundance trends that paralleled those of total ARGs across fish species and seasons. The dynamics of these ARGs correlated closely with the overall trends in total ARG abundance (C).

6.3 Associations between human pathogens and ARGs in cultured fishes

Given the high accumulation of human pathogens and the prevalence of ARGs in fish skins and guts, we conducted network analysis to elucidate their interactions and to identify potential pathogenic hosts of ARGs with significant implications for human health. The results showed robust associations between pathogens and ARGs in omnivorous and herbivorous skins (Figure 6-14A and B) and guts (Figure 6-15A and B), whereas such associations were absent in carnivorous species. *Staphylococcus aureus* and *K. pneumoniae* may be the key ARG hosts due to their strong associations with multiple ARGs and their prevalence in these fishes (Figure 6-14A and 6-15B). *Escherichia coli* was prominently linked to ARGs in the guts of herbivorous species (Figure 6-15B). The identification of these resistant pathogens highlights the health risks of handling and consuming farmed seafood. Additionally, the diversity of ARG-associated human pathogens increased from omnivorous to herbivorous fish in both skins and guts, with the highest level observed in herbivorous fish guts (Figure 6-14C). This suggests herbivorous fish as a critical reservoir of resistant pathogens.

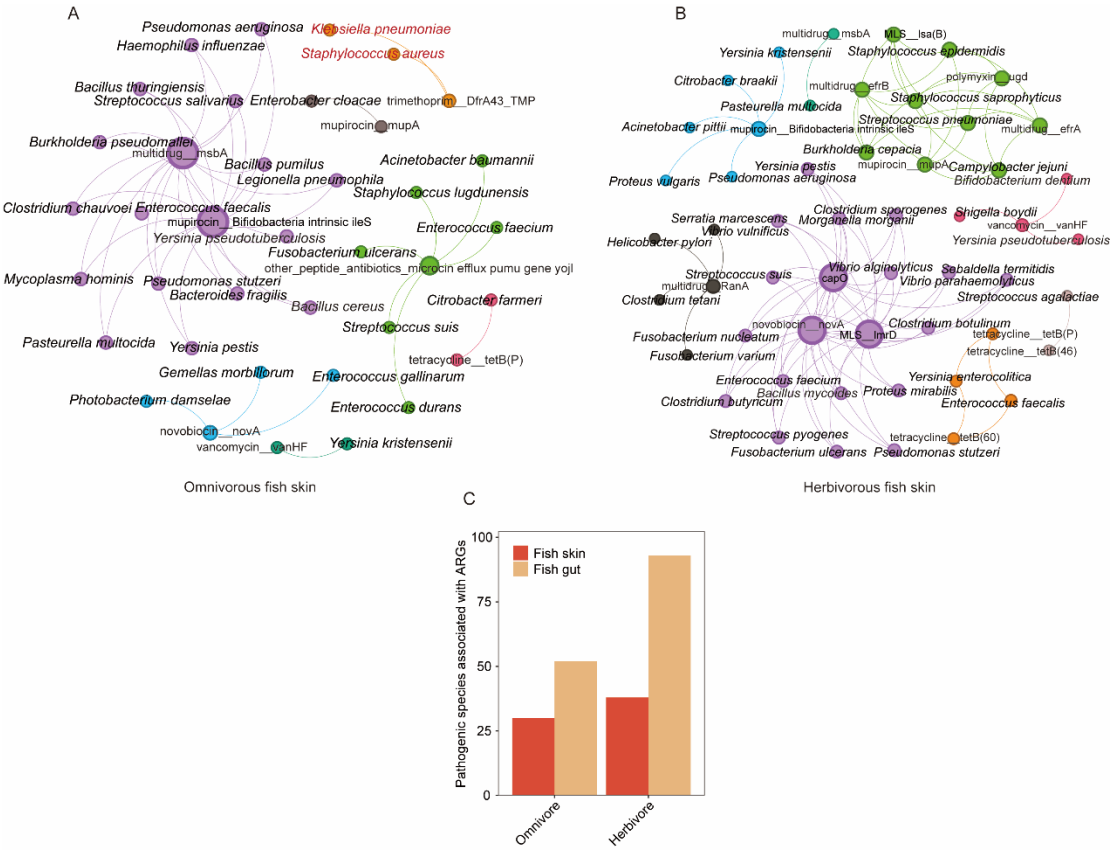

Figure 6-14 Human pathogens had close associations with antibiotic resistance genes (ARGs) in omnivore (A) and herbivore (B) skins. Italic letters denote pathogenic species. Regular letters represent the ARG subtype. The red color highlights the discriminative contaminants. The node size increases with the number of edges, which represent a significantly positive Spearman's rank correlation ($r \geqslant 0.7$ and $p < 0.05$). The diversity of ARG-associated human pathogens increased from omnivorous to herbivorous species in both skins and guts (C).

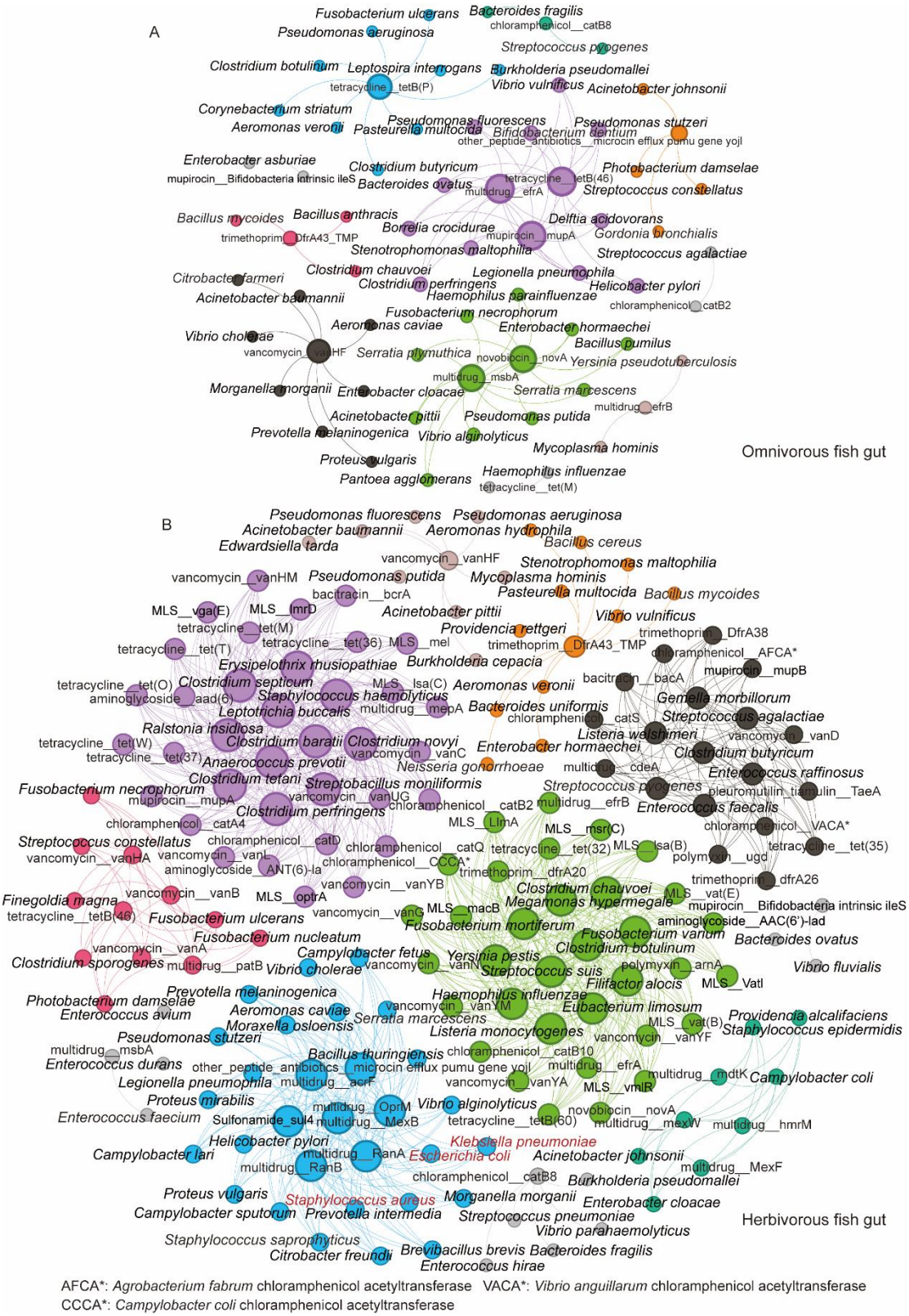

Figure 6-15 Human pathogens had close associations with antibiotic resistance genes (ARGs) in omnivore (A) and herbivore (B) guts. Italic letters denote pathogenic species. Regular letters represent ARG subtypes. The red color highlights the discriminative

contaminants. The node size increases with the number of edges, which represent a significantly positive Spearman's rank correlation ($r \geqslant 0.7$ and $p < 0.05$).

6.4 Source dynamics of human pathogens, resistomes, and resistant pathogens in cultured fishes

Using the above read-based metagenomic results, we applied FEAST to quantify the contributions of putative sources (including seawater, sediments, and feeds) to human pathogens and resistomes in cultured fishes. We found that the unknown source played a dominant role in shaping the pathogen profiles in fish skins and guts, followed by seawater, sediments, and feeds (Figure 6-16A). Comparison of the source profiles across fish species with different dietary behaviors highlighted this finding, showing the largest proportion of pathogen contributions originated from the unknown source across all feeding behaviors (Figure 6-16B). Its contribution was generally elevated in the skins and guts of omnivorous and herbivorous species relative to carnivorous species and exhibited contrasting seasonal trends in omnivorous and herbivorous guts (Figure 6-16B). These patterns align closely with the distribution of pathogen abundance across fish species and seasons, suggesting that the dynamics of human pathogens were driven by this source. According to the elevated levels of dominant pathogens observed in fish tissues compared with the environment and feed, the unknown source may be associated with the explosive proliferation of these pathogens. In contrast to the pathogen source dynamics, the unknown source had a dramatically lower contribution to the resistome profiles, comprising less than 25% of the total

(Figure 6-16C). This decline was likely attributed to the considerably lower levels of resistant bacteria compared to pathogenic species (Figure 6-17A), which constrained their dissemination and proliferation, as well as the resistomes carried by them, into fish bodies. Seawater became the predominant source, contributing to over 45% of the resistomes from the sources (Figure 6-16C). Feeds developed into a significant contributor to fish resistomes, accounting for a large proportion in source profiles (Figure 6-16C). Investigation of the source profiles across fish species with different feeding behaviors revealed fluctuations across the fish type and season (Figure 6-16D). These variations were more complex than the trends in relative abundance. The seasonal pattern within each fish type was inconsistent even with the relatively stable ARG abundance in carnivorous and omnivorous species. This suggests that, beyond the source dynamics, additional factors may significantly influence the resistome patterns in cultured fishes. Focusing on the source profiles of herbivorous fish species across seasons, we observed a higher proportion from seawater, sediment, and trash fish (Feed-TF) in both skins and guts during summer compared with winter (Figure 6-16D). Among these, trash fish used as feed during summer contained a substantially higher concentration of resistomes than feed pellets used in winter (Figure 6-17B). Its contribution may be partially responsible for the increased ARG abundance in herbivorous skin and guts during this season.

Due to the lack of metagenome-assembled data on ARG pathogenic hosts in cultured fishes, we used Venn diagrams to profile source linkages by identifying the overlaps

between ARG-associated pathogenic species in cultured fishes and pathogenic hosts detected from assembled contigs in putative sources. Among ARG-associated pathogenic species in cultured fish skins and guts, 31% (18/58) and 28% (30/107), respectively, overlapped with the pathogenic hosts of ARGs from seawater, sediment, and feed (trash fish) (Figure 6-18A and B). Most of these shared species were prevalent in both omnivorous and herbivorous fish species (Table 6-1). Among the identified sources, feed contributed the highest proportion of these pathogenic hosts in both skins and guts (Figure 6-18A and B), including *K. pneumoniae* (Navon-Venezia et al., 2017), *E. coli* (Poirel et al., 2018), *Enterobacter cloacae* (multidrug resistance) (Mezzatesta et al., 2012), *Streptococcus suis* (Palmieri et al., 2011), and *V. parahaemolyticus* (Elmahdi et al., 2016), which do not naturally occur in fish microbiomes (Figure 6-18C and D). This result highlights that feed introduced foreign resistant pathogens into the mariculture systems and thus represents a critical source of these harmful bacteria in cultured fishes. Compared with omnivorous species, herbivorous species exhibited a higher diversity of ARG-associated pathogenic species shared with seawater and feed, particularly in gut samples (Figure 6-19A – D). More ARG-associated pathogenic species may be introduced into herbivorous fish guts from these sources.

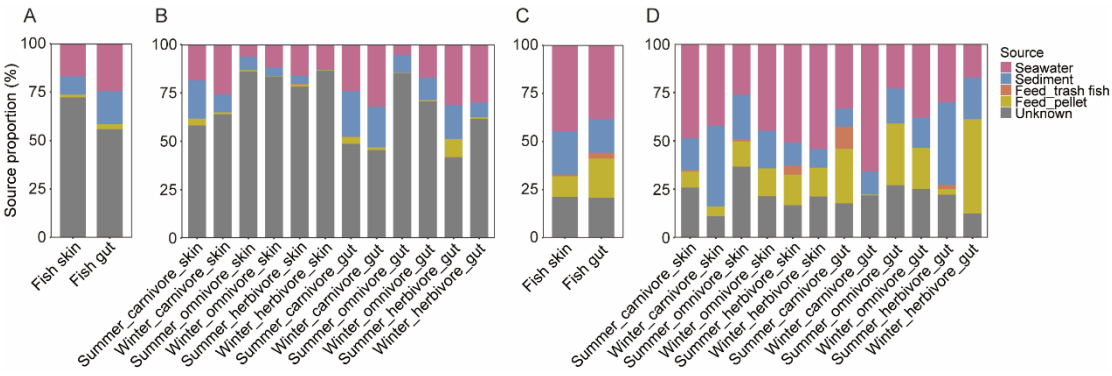

Figure 6-16 Source dynamics of human pathogens and resistomes in cultured fishes. Seasonal source profiles of human pathogens (A) and resistomes (C) in skins and guts. Source profiles of human pathogens (B) and resistomes (D) in cultured fishes with different feeding behaviors.

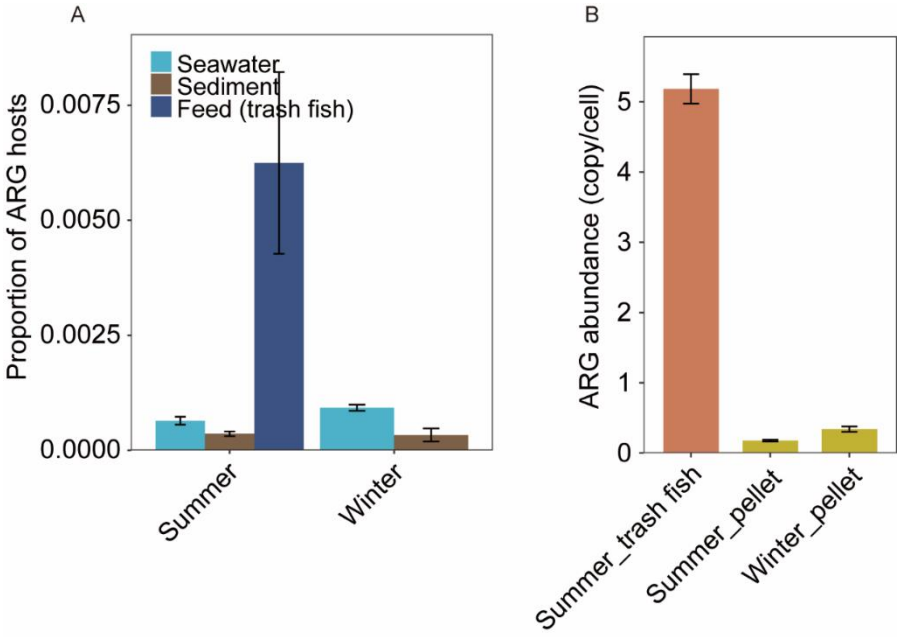

Figure 6-17 Antibiotic resistance gene (ARG) hosts in environments and feeds constituted a small proportion (≤ 1%) of the total bacterial population (A). Trash fish used as feed during summer contained a substantially higher concentration of resistomes than feed pellets used in winter (B).

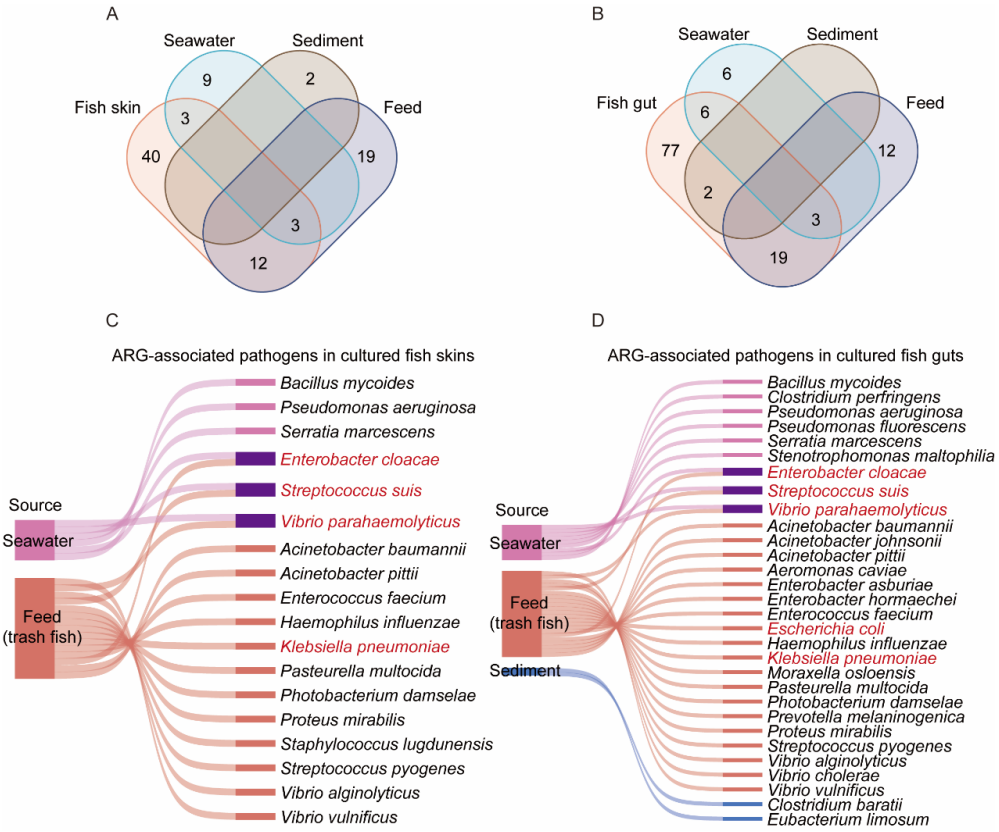

Figure 6-18 Pathogenic species associated with antibiotic resistance genes (ARGs) identified in cultured omnivorous and herbivorous fish skins and guts were shared with potential pathogenic hosts of ARGs in seawater, sediment, and feed (trash fish).

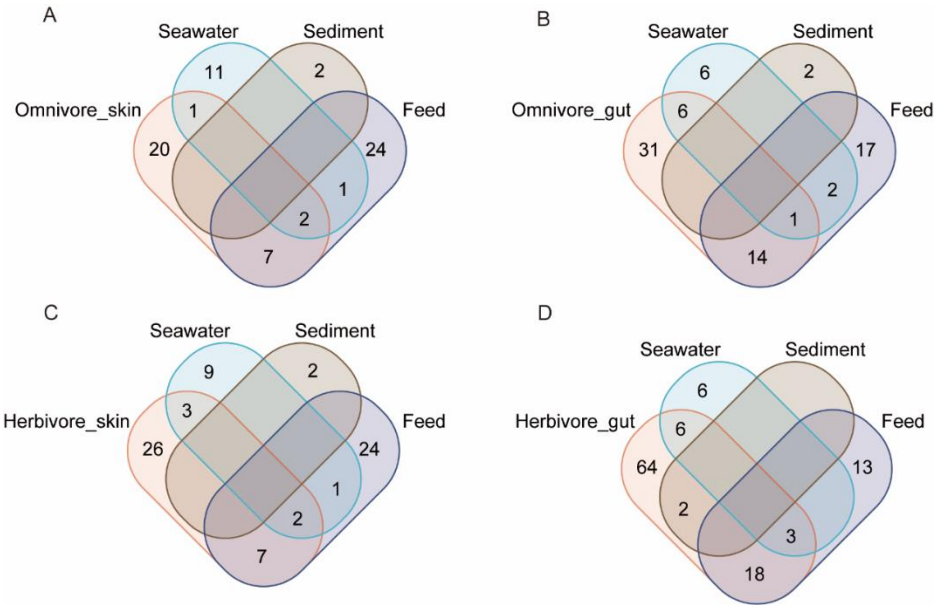

Figure 6-19 Compared with omnivorous species, herbivorous species exhibited a higher

diversity of antibiotic resistance gene (ARG)-associated pathogenic species shared with seawater and feed, particularly in gut samples.

Table 6-1 The potential pathogenic hosts of antibiotic resistance gene (ARGs) in cultured fishes from seawater, sediment, and feed (trash fish).

Bacterial species	Source	Omnivore		Herbivore	
		Skins	Guts	Skins	Guts
Bacillus mycoides	Seawater	NA	Yes	Yes	Yes
Clostridium perfringens	Seawater	NA	Yes	NA	Yes
Pseudomonas aeruginosa	Seawater	Yes	Yes	Yes	Yes
Pseudomonas fluorescens	Seawater	NA	Yes	NA	Yes
Serratia marcescens	Seawater	NA	Yes	Yes	Yes
Stenotrophomonas maltophilia	Seawater	NA	Yes	NA	Yes
Enterobacter cloacae	Seawater; feed	Yes	Yes	NA	Yes
Streptococcus suis	Seawater; feed	Yes	NA	Yes	Yes
Vibrio parahaemolyticus	Seawater; feed	NA	NA	Yes	Yes
Acinetobacter baumannii	Feed	Yes	Yes	NA	Yes
Acinetobacter johnsonii	Feed	NA	Yes	NA	Yes
Acinetobacter pittii	Feed	NA	Yes	Yes	Yes
Aeromonas caviae	Feed	NA	Yes	NA	Yes
Enterobacter asburiae	Feed	NA	Yes	NA	NA
Enterobacter hormaechei	Feed	NA	Yes	NA	Yes
Enterococcus faecium	Feed	Yes	NA	Yes	Yes
Escherichia coli	Feed	NA	NA	NA	Yes
Haemophilus influenzae	Feed	Yes	Yes	NA	Yes
Klebsiella pneumoniae	Feed	Yes	NA	NA	Yes
Moraxella osloensis	Feed	NA	NA	NA	Yes
Pasteurella multocida	Feed	Yes	Yes	Yes	Yes
Photobacterium damselae	Feed	Yes	Yes	NA	Yes
Prevotella melaninogenica	Feed	NA	Yes	NA	Yes
Proteus mirabilis	Feed	NA	NA	Yes	Yes
Staphylococcus lugdunensis	Feed	Yes	NA	NA	NA
Streptococcus pyogenes	Feed	NA	Yes	Yes	Yes
Vibrio alginolyticus	Feed	NA	Yes	Yes	Yes
Vibrio cholerae	Feed	NA	Yes	NA	Yes
Vibrio vulnificus	Feed	NA	Yes	Yes	Yes
Clostridium baratii	Sediment	NA	NA	NA	Yes
Eubacterium limosum	Sediment	NA	NA	NA	Yes

NA, not applicable.

6.5 Transmission of ARGs from the environment and feed to cultured fishes

To better understand the impact of ARG transfer on the accumulation of typical ARGs and the evolution of human pathogens in cultured fishes, we characterized the mobile ARGs detected from the assembled contigs in putative sources and examined their overlaps with the enriched ARGs and pathogen-associated ARGs in cultured fishes. The results showed no overlap between the enriched ARGs in cultured fishes and mobile ARGs from putative sources, suggesting that ARG transfer may have a limited influence on ARG accumulation in cultured fishes. However, 19.3% (16/83) of the pathogen-associated ARGs in cultured fishes were shared with mobile ARGs in seawater and feed (trash fish) (Figure 6-20A). These genes included *OprM*, *RanA*, *RanB*, *MexB*, and *acrF*, which were strongly associated with the key resistant pathogens (e.g., *S. aureus*, *K. pneumoniae*, and *E. coli*) in herbivorous fish guts (Figure 6-15B and 6-20B). These results suggest significant transmission of ARGs from seawater and feed to fish, contributing to the restructuring of resistance profiles in human-associated pathogens. Feed may exert a greater influence on ARG transmission compared with seawater due to its higher contribution (Figure 6-20B). Notably, 55.6% (5/9) of the seawater mobile ARG subtypes overlapped with those in feeds (Figure 6-20A), further reflecting that feed acted as an important source to spread mobile resistome into the mariculture system. The number of shared mobile ARG subtypes increased from omnivorous to herbivorous fish in both skins and guts, with the highest diversity observed in herbivorous fish guts (Figure 6-20C). These results indicate that ARG transmission from environmental and feed sources was more efficient in herbivorous

fish, particularly in their guts. Further analysis of MGEs revealed that over 95% of mobile ARGs in seawater were plasmid-mediated, in contrast to only approximately 25% observed in feed (Figure 6-20D). Phage-associated ARGs increased significantly from seawater to feeds, accounting for over 50% of the total mobile resistomes in feeds (Figure 6-20D). Plasmids were the dominant vectors of ARG transmission in seawater, while phages played a more significant role in feed.

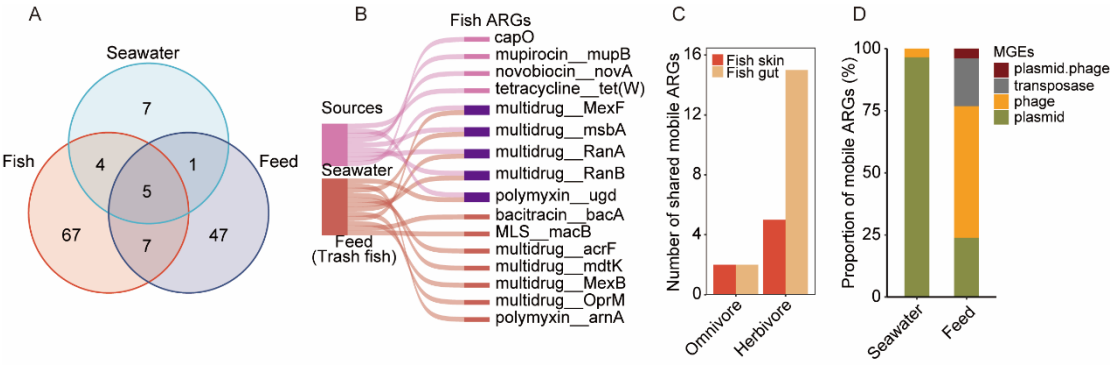

Figure 6-20 Antibiotic resistance genes (ARGs) associated with human pathogens in cultured fishes were shared with those mobile in seawater and feed (trash fish) (A and B). The number of these ARGs increased from omnivorous to herbivorous species in both skins and guts (C). Resistome transmission mechanisms were different between seawater and feed (trash fish) (D).

6.6 Seafood contamination associated with environmental changes, source dynamics, and ARG transmission

Mariculture zones, heavily influenced by anthropogenic activities, have become critical hotspots for the proliferation of human pathogens and genetic materials over recent

decades (Watts et al., 2017). We found that natural bacteriomes and resistomes in the mariculture environment were substantially impacted by farming activities. The structure of human pathogens and resistomes, as revealed by read-based metagenomic analysis, in the mariculture system exhibited significant divergence from those in healthy oceanic systems (Figure 6-21A and B, PERMANOVA, $p_{pathogens} < 0.001$, $p_{resistome} < 0.001$). Many human pathogens and ARGs were notably elevated in mariculture seawater compared with healthy oceanic systems (LEfSe, LDA score > 2, $p < 0.05$) and overlapped with those enriched in fish samples (Figure 6-22A and B). These results indicate that environmental shifts in human pathogens and resistomes likely enhanced their exposure to cultured fishes, leading to their dissemination, accumulation and reconstruction in fish tissues. We found that cultured carnivorous and omnivorous/herbivorous species, as well as cultured SC, displayed substantial differences in the gut microbiota compared with wild-caught counterparts from Hong Kong coastal waters (Huang et al., 2020) (Table 6-2). Anthropogenically associated pathogens, such as *S. aureus*, *K. pneumoniae*, and *E. coli*, became core constituents in both gut and skin microbiomes of cultured fishes (Figure 6-23A and B). These observations align with a recent study demonstrating that farm environmental exposure induces compositional alterations and pathogen enrichment in human gut microbiomes (Sun et al., 2020). Although limited data on ARGs in wild-caught fishes from Hong Kong coastal waters preclude direct comparisons, previous research from coastal New England indicates that the prevalence and relative abundance of these typical ARGs in wild fishes vary with environmental changes (Korry & Belenky, 2023). This suggests

that the elevated concentrations in the mariculture environment could facilitate their accumulation in cultured fishes. Source attribution analysis further substantiated these findings, identifying substantial contributions from environmental sources to pathogen and resistome profiles in cultured fishes. However, the source tracking results also implied that additional mariculture-related factors were critical in shaping these profiles, as evidenced by the contributions of the unknown source to pathogen communities and feed sources to resistomes discussed above.

In pollution-free natural ecosystems, the environments and diets are the key contributors to intrinsic human pathogens in wild fishes (Belkin & Colwell, 2006). However, the introduction of farming stressors and the concomitant increase in human-associated pathogens in mariculture systems may have disrupted these natural source dynamics. Our source tracking analysis revealed that the unknown source related to the explosive growth of human-associated pathogens was the key driver, rather than environmental and feed sources. This pathogen bloom and the resultant shift in pathogen dynamics may be linked to mariculture-induced suppression of critical immune functions in fish, encompassing pathobiological traits, immunogenetic profiles, and physiological defenses that are essential for maintaining health and resisting pathogen invasion in wildlife (Ellis, 2001). Researchers have shown that mariculture-associated stressors, such as intensive practices, controlled feeding regimens, and poor water quality, can undermine these protective mechanisms and render fishes more susceptible to environmental human pathogens (Endo et al., 2002; Magnadóttir, 2006).

In this case, the increase in *S. aureus*, *K. pneumoniae*, and *E. coli* in mariculture seawater could facilitate their successful invasion into fish bodies, leading to the colonization and enrichment of these pathogens in cultured fishes. Furthermore, changes in dynamics may alter the seasonal variation patterns in pathogen profiles of cultured fishes, as indicated by the consistent seasonal trends in the proportions of the unknown source and the corresponding pathogen abundance.

Feed is extensively employed in mariculture systems to optimize yield by promoting animal growth. Recent studies have identified fishmeal as a reservoir of diverse and abundant ARGs and human pathogenic bacteria (Han et al., 2017, 2019). Its widespread application has been linked to the propagation of ARGs in mariculture environments (Han et al., 2017). Our findings corroborate these observations and further establish feed as an important contributor to fish resistomes and resistant human pathogens. The use of trash fish has introduced novel pathogenic hosts and mobile ARGs into environmental compartments, influencing the resistant pathogens and their carrying resistance profiles in cultured fishes. Additionally, trash fish contributed to the seasonal increase of resistomes in herbivorous fish organs during summer and amplified the prevalence of ARG-associated pathogens, particularly the rarely observed emerging and foodborne pathogenic hosts (e.g., *K. pneumoniae* and *E. coli*), and typical ARGs in fish bodies via source input (Figures 6-18 and 6-22B). The introduction of these harmful elements may decrease the fish immune system as seen in rats (Yang et al., 2017) and other fish species (Wu et al., 2021) and further favored the enrichment of

human pathogens and typical ARGs. For example, infection by drug-resistant *K. pneumoniae* has been reported to suppress the immune cells and cause tissue inflammation in the fish species *Lepisosteus oculatus* (Wu et al., 2021). These results underscore that feed applications, particularly the use of trash fish, serve as a significant contamination source and a crucial driver for the reconstruction of resistomes, resistant pathogens, and even human pathogens in mariculture systems and cultured fishes. Hence, there is an urgent need for a comprehensive re-evaluation of feed practices to mitigate associated health risks and to ensure the long-term sustainability of mariculture. Interspecies differences in microbial structure and ARG profiles have been well documented in the skins and guts of wild fishes (Korry & Belenky, 2023; Ross et al., 2019). These differences have often been attributed to variations in environmental microbial communities and dietary preferences across species. However, such variability is considerably diminished in cultured fishes. Environmental homogeneity, coupled with the continuous provision of anthropogenic diets, limits the diversity of microbial and resistome sources available to cultured fishes (Dhanasiri et al., 2011; Yukgehnaish et al., 2020). Furthermore, immunosuppression facilitates the invasion and proliferation of biological elements from environmental compartments and feeds. These combined influences lead to a convergence in the source dynamics of human pathogens and resistomes, promoting their homogenization across different cultured fish species. This phenomenon is evident based on the similar source profiles of human pathogens across a broad range of cultured fish species. Nonetheless, resistome variation among species showed greater conservatism in interspecies differences and

source dynamics. This may be due to the stringent conditions required for ARG dissemination from environmental and feed sources into fish bodies, such as the necessity of strong selection pressures, MGEs as transmission vectors, and the ability to overcome phylogenetic barriers between bacterial hosts (Partridge Sally et al., 2018). Of note, the concentration of both human pathogens and resistomes displayed an increasing trend from carnivorous to herbivorous fish, contrasting with patterns observed in wild fishes with different feeding behaviors or trophic levels (Korry & Belenky, 2023). This reversal in cultured fishes may be attributable to species-specific immunosuppression and feed application. Herbivorous and omnivorous species have evolved weaker immune defenses, such as thinner mucosal barriers (Gomez et al., 2013), which render them more susceptible to pollutant invasion and proliferation. Consequently, human pathogens, ARGs, and ARG-associated pathogens originating from environmental contamination and feed application may spread through seawater across cages and disproportionately affect these species, even without direct ingestion.

HGT mediated by MGEs is a critical mechanism by which human pathogens acquire or exchange ARGs from other microbiota (Partridge Sally et al., 2018). While ARGs in pristine deep-sea environments exhibit low transfer potential (Collins et al., 2021), those in farming-associated coastal systems display significantly higher mobility (Muziasari et al., 2016; Watts et al., 2017). We identified a diverse array of ARG subtypes co-located with MGEs in both seawater and feed, particularly on plasmids and phages. These mobile ARGs corresponded to a substantial proportion of pathogen-

associated ARGs identified in cultured fishes, suggesting their high mobility may promote transmission from environmental and feed sources to cultured fishes, thereby reshaping pathogen resistance profiles. Notably, *S. aureus*, *K. pneumoniae*, and *E. coli* in herbivorous fish guts presented an ARG profile that is not commonly found in wild fish species at low trophic levels (Korry & Belenky, 2023). These ARGs overlapped with those identified in mobile resistomes from seawater and feed, indicating that ARG transmission actively supported the acquisition or exchange of resistance traits in these pathogens. Moreover, the transmission of ARGs likely contributed to the elevated levels of resistomes and resistant human pathogens in herbivorous fish, where ARG transfer from the environmental compartment and feed was more pronounced. Collectively, these findings underscore the prevalence of ARG transmission in mariculture systems, where it accelerates the evolution of resistance and resistant pathogens in farmed fishes.

In summary, farming-induced alterations in environmental concentrations of human pathogens and antimicrobial resistomes, coupled with their enhanced dissemination from the environment and feed to cultured fish via source dynamics and HGT, facilitated the enrichment and reconstruction of these biological elements in cultured fishes. This process may exacerbate health risks for coastal populations by increasing exposure to resistant pathogens during seafood processing and consumption.

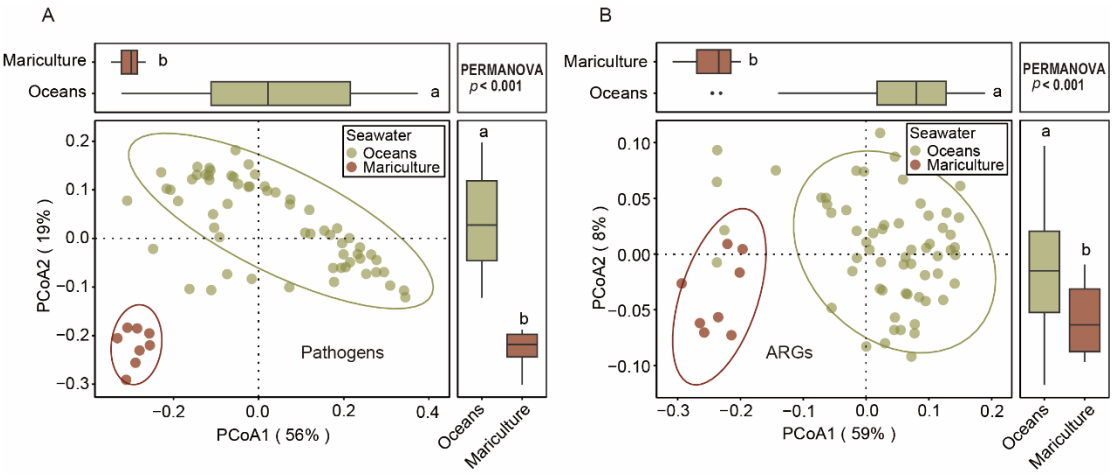

Figure 6-21 Structural compositions of human pathogens (A) and resistomes (B) in the mariculture system were significantly different from those in the healthy oceanic systems. PERMANOVA, permutational multivariate analysis of variance.

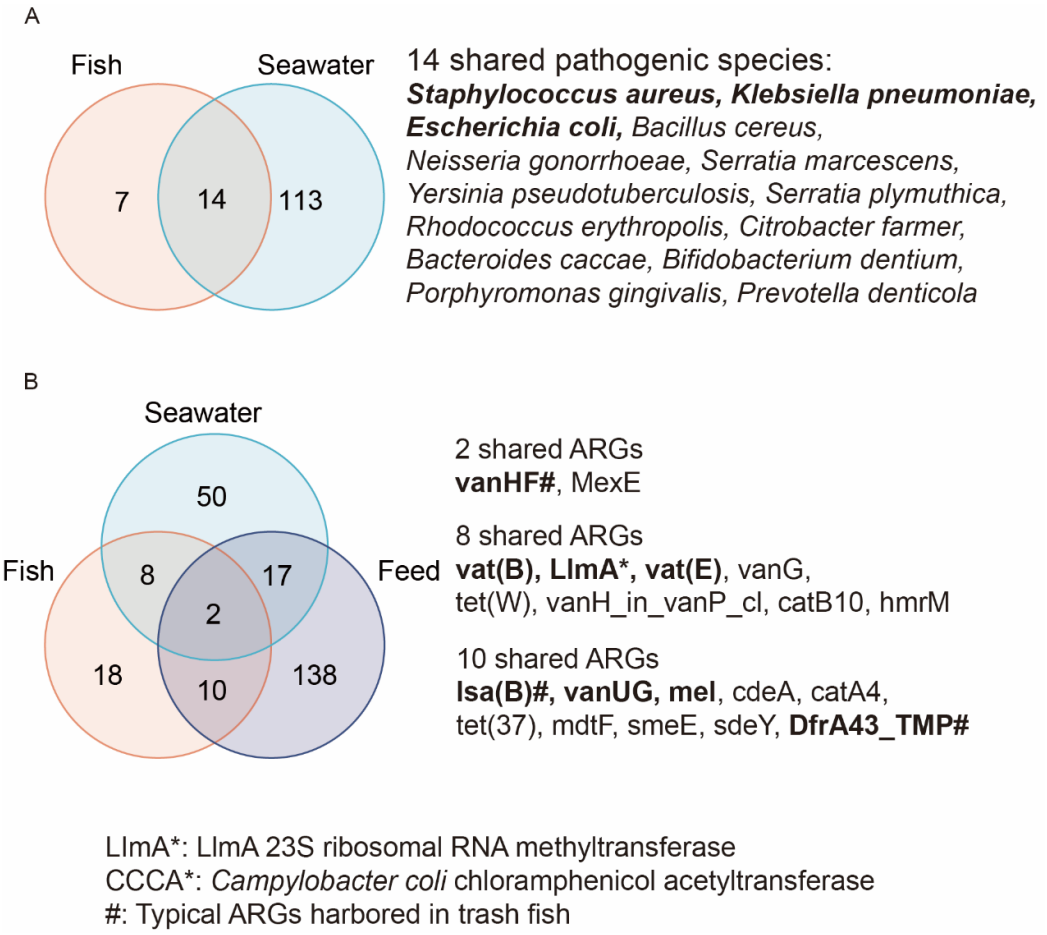

Figure 6-22 Human pathogens and antibiotic resistance genes (ARGs) elevated in

mariculture seawater compared with healthy oceanic systems (linear discriminant analysis effect size, linear discriminant analysis score > 2, $p < 0.05$) and overlapped with those enriched in the fish samples (A and B).

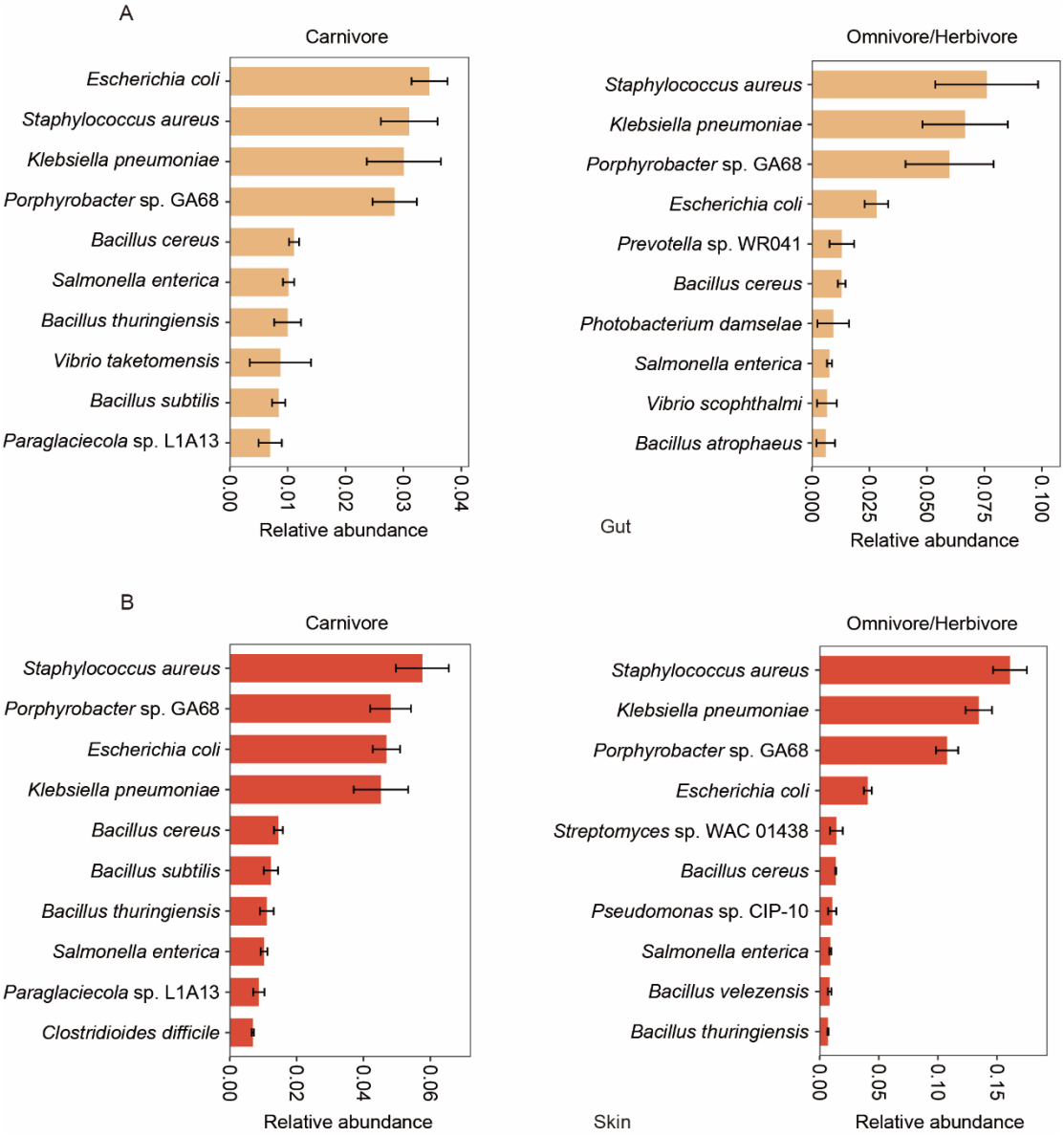

Figure 6-23 Anthropogenically associated pathogens, such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*, were core constituents in the gut and skin microbiomes of cultured fishes (A and B).

Table 6-2 Substantial differences in the gut core bacteria between cultured and wild-caught fish species.

Feeding behavior or species	Gut core bacteria	Mariculture	Wild-caught
Carnivore	*Escherichia coli*	Yes	No
Carnivore	*Staphylococcus aureus*	Yes	No
Carnivore	*Klebsiella pneumoniae*	Yes	No
Carnivore	*Porphyrobacter* sp. GA68	Yes	No
Carnivore	*Bacillus cereus*	Yes	No
Carnivore	*Salmonella enterica*	Yes	No
Carnivore	*Bacillus thuringiensis*	Yes	No
Carnivore	*Vibrio taketomensis*	Yes	No
Carnivore	*Bacillus subtilis*	Yes	No
Carnivore	*Paraglaciecola* sp. L1A13	Yes	No
Omnivore/Herbivore	*Staphylococcus aureus*	Yes	No
Omnivore/Herbivore	*Klebsiella pneumoniae*	Yes	No
Omnivore/Herbivore	*Porphyrobacter* sp. GA68	Yes	No
Omnivore/Herbivore	*Escherichia coli*	Yes	No
Omnivore/Herbivore	*Prevotella* sp. WR041	Yes	No
Omnivore/Herbivore	*Bacillus cereus*	Yes	No
Omnivore/Herbivore	*Photobacterium damselae*	Yes	No
Omnivore/Herbivore	*Salmonella enterica*	Yes	No
Omnivore/Herbivore	*Vibrio scophthalmi*	Yes	No
Omnivore/Herbivore	*Bacillus atrophaeus*	Yes	No
Siganus canaliculatus	*Klebsiella pneumoniae*	Yes	No
Siganus canaliculatus	*Staphylococcus aureus*	Yes	No
Siganus canaliculatus	*Escherichia coli*	Yes	No
Siganus canaliculatus	*Photobacterium damselae*	Yes	No
Siganus canaliculatus	*Porphyrobacter* sp. GA68	Yes	No
Siganus canaliculatus	*Vibrio scophthalmi*	Yes	No
Siganus canaliculatus	*Bacillus cereus*	Yes	No
Siganus canaliculatus	*Streptomyces* sp. WAC 01438	Yes	No
Siganus canaliculatus	*Salmonella enterica*	Yes	No
Siganus canaliculatus	*Clostridioides difficile*	Yes	No

6.7 Phylogenetic evidence for seafood-borne community disease outbreaks

Since the 1960s, the epidemiology of MRSA has evolved into a global clinical threat due to its high morbidity and mortality, with difficulty in treatment for the resistance to

many classes of antibiotics (Lee et al., 2018; Turner et al., 2019). Our surveillance of antibiotic-resistant human pathogens in cultured fishes isolated a ciprofloxacin-resistant strain of *S. aureus* from the edible tissues of these fish species (Figure 6-24A). Phylogenetic assessment clustered it closely with a MRSA strain (*S. aureus* O14S_SA) previously isolated from the skin of schoolchildren with impetigo and scabies (Taiaroa et al., 2021) (Figure 6-24B and C). These clustered strains exhibited similar resistance profiles, differing by only one resistance type (Figure 6-24D). The isolated strain also carried a featural resistance gene of MRSA, *blaZ*, which can encode beta-lactamase PC1 and confer resistance to penicillin antibiotics (Alexander et al., 2023) (Figure 6-24D). Collectively, these results confirm that the isolated strain is MRSA. The presence of this 'superbug' further provides phenotypic evidence for the emergence of human pathogens and antibiotic resistance in farmed fishes. The current mariculture process may be beneficial for the evolution of commensal bacteria and the enrichment of resistant human pathogens. This poses a formidable clinical threat to coastal communities via seafood consumption or other seafood-related pathways. Indeed, community-based infections caused by MRSA have long been a public health issue in Hong Kong (Ho et al., 2008). The detection of this strain in cultured fishes raises concerns that community-associated outbreaks of MRSA might be linked to seafood processing and consumption, which has been largely overlooked in previous studies (Boost et al., 2013). This concern has been supported by the freshwater aquaculture cases, where invasive group B *Streptococcus* (ST283) from freshwater fishes caused over 77 infections in Hong Kong between 2021 and 2024, primarily related to handling

or contact with raw fishes (Kong, 2024). It highlights that cultured seafood might be an important transmission vector of MRSA and other resistant pathogens from mariculture systems to consumers, contributing to community-based infections in coastal regions. These findings underscore the urgent need for greater attention to mariculture-derived infections and their associated transmission dynamics of resistant pathogens from farms to people in future research.

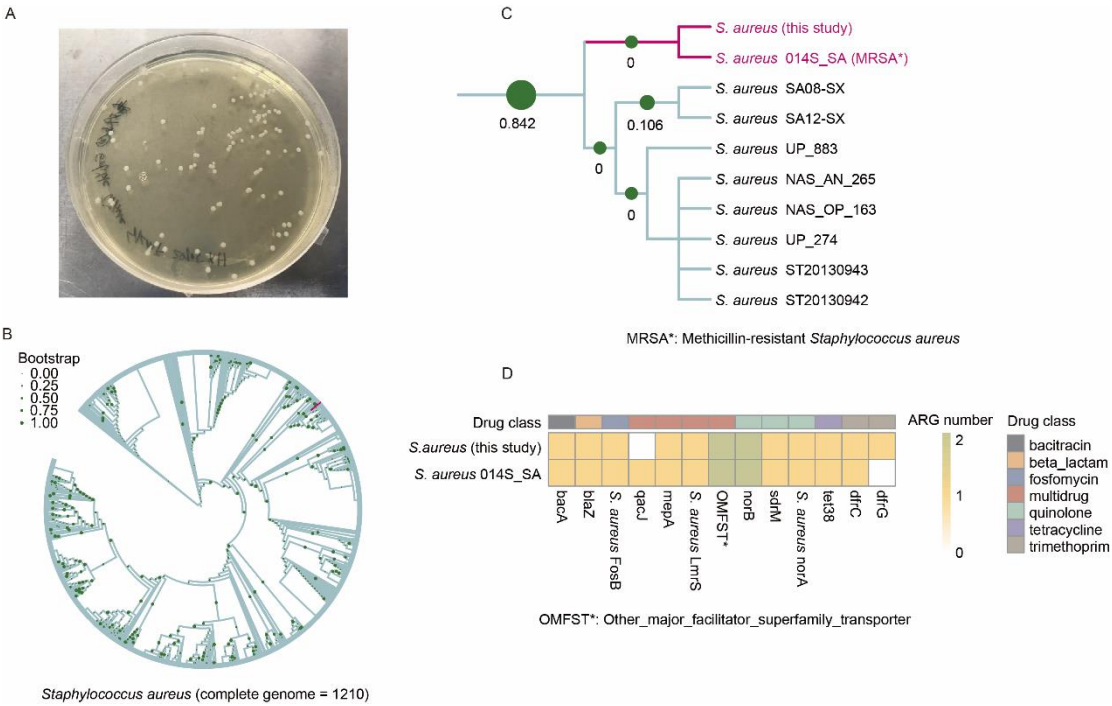

Figure 6-24 A ciprofloxacin-resistant strain of *Staphylococcus aureus* was isolated from the fish edible tissues (A). Phylogenetic assessment (B) clustered it closely with a MRSA strain (*Staphylococcus aureus* O14S_SA) previously isolated from the skin of schoolchildren with impetigo and scabies (C). These strains almost exhibited the same multiple resistance except one resistant type (D).

6.8 Summary

In response to the elevated levels of human pathogens, resistomes, and ARG hosts identified in mariculture zones in Chapter 5, we conducted detailed metagenomic analysis to elucidate their enrichment in cultured fishes and the underlying mechanisms associated with farming-induced environmental alterations and performed culture-based surveillance of resistant pathogens in these fish species to assess potential health implications for coastal communities. The key findings of this chapter are summarized below.

1. Various pathogenic species were identified within the mariculture system and in the cultured fishes. The pathogen levels in cultured fish skins and guts were significantly elevated compared with those in surrounding environments and healthy oceanic systems, indicating the substantial pathogen enrichment in cultured seafood. This accumulation involved a sharp increase in emerging and foodborne pathogens, such as *S. aureus*, *K. pneumonias*, and *E. coli*. Relative to wild fishes, cultured fishes exhibited a reduced interspecies variation in the structural composition of human pathogens, coupled with a marked increase in their relative abundance from carnivorous to herbivorous fish species.

2. A broad spectrum of resistomes with a similar dominance of resistance profiles was prevalent in the mariculture system and cultured fishes. Typical resistance genes were notably enriched in cultured fishes, with the dominance of *vanHF, vanUG, vat(B),*

lsa(B), LlmA 23S ribosomal RNA methyltransferase, *vat(E), mel, DfrA43_TMP, catD*, and *C. coli* chloramphenicol acetyltransferase. Nevertheless, cultured fishes showed reduced interspecies variation in the resistome structure compared with wild fishes, with the relative abundance of resistance genes obviously increasing from carnivorous to herbivorous fish species.

3. Strong associations were observed between human pathogens and ARGs in the skins and guts of omnivorous and herbivorous species, but not in carnivorous species. The diversity of ARG-associated human pathogens increased from omnivorous to herbivorous species. Emerging and foodborne pathogens appear to be the key hosts of ARGs due to their strong associations with multiple ARGs and their prevalence in these fishes. The occurrence of these resistant pathogens exacerbated the health risks associated with seafood processing and consumption. It is noteworthy that herbivorous fish guts harbored the highest levels of pathogenic species associated with ARGs, positioning them as the severe reservoir of resistant pathogens.

4. Source dynamics between fish human pathogens and resistomes were significantly different. Environmental compartments and feed were identified as critical sources for fish resistomes and resistant pathogens, but they did not serve as determinants for pathogens. The accumulation of human pathogens was closely linked to their invasion and proliferation in cultured fishes, which was triggered by environmental changes and immunosuppression associated with mariculture practices. Feed applications were vital

in driving the seasonal variations in the relative abundance of resistomes in cultured fishes and in the development of special ARGs and resistant pathogens in omnivorous and herbivorous species.

5. Various ARGs associated with human pathogens in cultured fishes overlap with mobile ARGs in seawater and feed (trash fish), indicating frequent transmission of ARGs from these reservoirs to fish bodies. Farming practices have enhanced the transferability of resistomes in the mariculture system, facilitating the acquisition or exchange of resistance by key pathogens in cultured fishes, particularly those in herbivorous species. These biological processes could accelerate the enrichment and evolution of resistance and resistant pathogens in farmed fishes.

6. The ciprofloxacin-resistant strain of *S. aureus* isolated from the fish edible tissue exhibited a close phylogenetic relationship with MRSA associated with skin infections and shared a similar resistance profile. This result provides strong phenotypic evidence for the emergence of human pathogens and antimicrobial resistance in cultured fishes, as highlighted by the above metagenomic findings. The detection of MRSA strain in cultured fishes further implies the potential for seafood processing and consumption to act as significant pathways for community-based infections in Hong Kong, which has been largely overlooked in previous research. The discovery of biological contamination in cultured fishes highlights the urgent need for greater attention to mariculture-derived infections and their associated transmission dynamics of resistant

pathogens from farms to folks in future research.

In conclusion, the metagenomic and culture-based surveillance of human pathogens and antimicrobial resistance in various cultured fish species at a representative mariculture farm in the Hong Kong fish culture zone has demonstrated the accumulation and restructuring of these harmful elements in cultured fishes, particularly in omnivorous and herbivorous species, compared with wild-caught fishes. The emergence of these elements was driven by environmental changes and further amplified by the source dynamic shifts and enhanced ARG transfer induced by mariculture practices. The isolation of MRSA strain from edible fish tissue further provides phenotypic evidence for seafood contamination and highlights the health risks associated with processing and consuming cultured seafood. To ensure seafood quality, it is advisable to cease the practice of feeding trash fish to cultured fish due to its substantial role in the accumulation of typical ARGs. Furthermore, farming and consumption of omnivorous and herbivorous fish species should be reconsidered according to their pronounced enrichment of harmful elements. Our findings underscore the urgent need to expand the surveillance and source control in the mariculture industry on a broader (even worldwide) scale to mitigate global seafood safety issues.

Chater 7 Conclusions and recommendations

7.1 General summary and major results

This thesis has provided comprehensive metagenomic insights into the structure and variations of bacteriomes and resistomes in human-impacted coastal waters across two of China ' s most densely populated and economically developed cities using metagenomic approaches. Additionally, it has examined biological contamination of cultured fishes associated with farming-induced environmental changes in a representative mariculture system located in a coastal city based on metagenomic and cultured-based methods. The objectives were to elucidate the extent, mechanisms, and health risks of anthropogenic influences on coastal bacterial communities and antimicrobial resistance relative to oceanic baselines, as well as their association with seafood contamination, to implicate environmental health and seafood safety in coastal regions.

We compared metagenomic differences in the structure and variations of bacteriomes and resistomes between human-impacted coastal waters and minimally impacted surface oceanic systems. We evaluated human disturbances based on the proliferation of human-associated bacteria and ARGs, which significantly contributed to the observed microbial shifts (Chapter 4). We integrated correlation analysis, db-RDA, and source identification to uncover the relationships between the characteristics of bacteriomes and resistomes (from diversity to structure) and the influencing factors, thereby clarifying the primary drivers and key anthropogenic stressors. To assess health

risks posed by coastal microbial alterations, we assembled genome-resolved human pathogens with antimicrobial resistance and assessed their links to community disease outbreaks based on phylogenetic analysis with the clinical strains (Chapter 5). Finally, in a representative mariculture system, we performed metagenomic and culture-based surveillance of the enrichment and underlying mechanisms, and health implications of human pathogens and antimicrobial resistance in cultured fishes to elucidate the linkage between mariculture-induced environmental changes and seafood contamination (Chapter 6).

Collectively, this thesis has provided a comprehensive understanding of the anthropogenic impacts and the underlying factors driving microbial alterations in coastal waters and farmed seafood. We have highlighted the potential health risks of community-associated infections transmitted through waterborne and foodborne routes and offer actionable recommendations for mitigating contamination. These findings provide valuable insights to support stakeholders in addressing microbial contamination and protecting human health, framed within the One Health and Global Health paradigms. The principal findings and conclusions of this thesis are summarized as follows.

1. Surface oceans offshore presented inherently low levels of human pathogens and antimicrobial resistomes, supporting high bacterial diversity and limited potential for gene transfer. In contrast, coastal waters exhibited a significant reduction in bacterial

diversity and a dramatic increase in pathogen abundance alongside notable changes in ARGs: increased relative abundance, diversity, transferability, and co-occurrence with human pathogens. These shifts involved the enrichment of human-associated bacteria and resistomes in coastal environments, where ARGs exhibited increased transferability to human pathogens. Relative to natural baselines, 65%, 35%, and 75% of the core species within coastal bacteriomes, resistomes, and host bacteria, respectively, showed alterations. Notably, we observed that various human pathogens with mobile ARGs detected in coastal waters, such as *K. pneumoniae*, *E. coli*, and *S. aureus,* are among the priority resistant pathogens listed by the WHO, suggesting high exposure risks to coastal populations.

2. Seasonal and regional variations in the characteristics of bacteriomes and resistomes from diversity and structure were observed in human-impacted coastal waters, with seasonal fluctuations exceeding regional differences. Bacterial diversity peaked significantly during winter in both coastal waters, accompanied by a decline in the relative abundance of human pathogens. The ARG diversity, relative abundance, and host range were higher during winter in both coastal waters, particularly in Hong Kong coastal waters. The increased levels of human-associated bacteria and ARGs in winter, particularly in Hong Kong coastal waters, were responsible for the anthropogenic disturbances on the inherent variations of coastal bacteriomes and resistomes, shifting the regulation from natural processes towards an anthropogenically driven pattern.

3. Environmental and anthropogenic factors collectively shaped the current patterns of coastal bacteriomes and resistomes. However, biodiversity loss, core bacterial species shifts, human pathogen proliferation, and resistome emergence were more correlated with anthropogenic factors, including antibiotic selection pressure, fecal contamination, and anthropogenically enhanced ARG transfer. Their elevated levels in winter, particularly in Hong Kong coastal waters, largely contributed to the proliferation of human-associated bacteria and ARGs in coastal waters, thereby driving the adverse alterations in coastal bacteriomes and resistomes. Among these drivers, anthropogenically enhanced ARG transfer emerged as the most significant, followed by fecal contamination and antibiotic selection pressure. We identified WWTP discharge and contaminated riverine runoff as the primary origins of these factors in Hong Kong and Qingdao, respectively. Further phylogenetic assessment showed close relationships between coastal resistant pathogens and clinical strains, highlighting enhanced health risks of coastal microbial alterations, which were related to disease outbreaks in coastal communities via recreational activities.

4. Cultured fishes, particularly omnivorous and herbivorous species, were enriched with emerging and foodborne pathogens (including *S. aureus*, *K. pneumoniae*, and *E. coli*) and typical ARGs (conferring resistance to vancomycin, MLS antibiotics, trimethoprim, and chloramphenicol). In contrast to wild-catch fish, cultured ones exhibited a notable increase in the levels of human pathogens and resistomes from carnivorous to herbivorous species, with a large reduction in compositional differences

of these biological elements among species. There is a strong association between human pathogens and ARGs, suggesting the prevalence of resistant human pathogens in cultured fishes. The sources of fish human pathogens and antimicrobial resistance exhibited distinct dynamics, with environmental and feed sources critical for resistomes and resistant pathogens but not determinable for pathogen enrichment. Pathogen accumulation was primarily attributed to their invasion and proliferation in cultured fishes. ARG transmission became prevalent in mariculture systems under farming pressures, promoting rapid gene exchange between environmental bacteria and fish pathogens and accelerating pathogen evolution in cultured fishes. Environmental changes, source dynamic shifts, and enhanced ARG transmission caused by mariculture practices collectively shaped the changes in human pathogens and antimicrobial resistance in cultured fishes. The isolation of MRSA strain in edible fish tissues provides additional phenotypic evidence and indicates the emergence of these harmful elements, as well as the associated health risks of consuming such seafood. Mariculture practices appear to have reshaped the structure of these elements in cultured fishes, particularly omnivorous and herbivorous species, highlighting an urgent need for contamination control in the mariculture sector. We also recommend reducing the use of trash fish.

7.2 Limitations and future perspectives

This thesis has provided metagenomic insights into anthropogenic influences on coastal waters and cultured seafood from a biological perspective and highlights the resultant

health risks to coastal populations. By employing a comprehensive collection of sample types, NGS, and advanced bioinformatics approaches, we have offered a more profound and integrated examination of microbial alterations in human-associated coastal ecosystems. These approaches mark a departure from previous studies that were constrained by technological limitations and a narrow range of sample types, which restricted their scope to monitoring only a few elements. Nevertheless, there are still certain limitations in the current findings. The restrictions and relevant suggestions are briefly discussed below.

Anthropogenically impacted coastal waters have been demonstrated as 'hotpots' for the proliferation of antimicrobial resistance and resistant bacteria. Our findings align with previous research showing that human-influenced areas harbor higher levels of human pathogens and ARGs compared to less-impacted coastal sites (Blaak et al., 2014; Fresia et al., 2019; Gabashvili et al., 2022; Jo et al., 2021; Zhou et al., 2022). Key sources include sewage discharge, estuarine inputs, and marine aquaculture, as revealed by traditional methods (Zheng et al., 2021). While these studies support the robustness of our findings, the precise of our conclusions were limited by the restricted regional and seasonal coverage. Future validation across broader temporal and geographic scales (e.g., multi-cities and multi-seasons) will be essential to solidify our conclusions. This also applies to the pattern of seasonal and regional variations, as our results suggest seasonality exerted a stronger influence than regionality on coastal bacteriomes and resistomes, consistent with previous reports (Cuadrat et al., 2020; Fuhrman et al., 2015;

Gilbert et al., 2012; Sunagawa et al., 2015; Wang et al., 2020).

Another limitation of this study is that the findings are based on a single database, which might introduce bias. ARG sequences are inherently complex and diverse, often associated with mobile genetic elements, and frequently exhibit high sequence homology, all of which can increase the likelihood of mis-annotation. Similarly, current pathogen list enables broad-spectrum monitoring, but it can also produce false positives. To improve annotation reliability, reduce both false positives and false negatives, and mitigate biases arising from database differences, future studies should implement cross-validation using other databases. These efforts can provide additional support or correction for our conclusions.

On the other hand, the genetic-level mechanisms and evolutionary dynamics of bacterial communities and their resistomes in coastal environments remain poorly understood. Addressing this knowledge gap requires the development of precise methodologies that leverage advanced genetic techniques. Such research would deepen our evolutionary understanding of bacterial contamination in coastal ecosystems and clarify the pathways by which resistance emerges and spreads.

Phylogenetic analysis revealed significant health risks associated with the potential re-exposure of coastal populations to antibiotic-resistant pathogens in human-associated coastal waters and cultured seafood. However, there is limited concrete evidence

regarding the viability and persistence of these pathogens in seawater and their dissemination to humans through waterborne pathways. Further research is required to characterize the resistance phenotypes of these pathogens *in situ*. Additionally, the development of advanced surveillance models is recommended to monitor the environmental behaviors and transmission routes of harmful elements from coastal systems and seafood to human populations. Such models would help clarify the dissemination pathways and underlying mechanisms, enabling targeted strategies to mitigate spread and protect human health within the One Health framework.

Meanwhile, the accumulation of harmful elements and the isolation of MRSA strain from cultured fishes underscores potential health risks associated with seafood consumption. However, since bacterial culture experiments involved enrichment, the actual concentration of MRSA in cultured fishes could not be determined, limiting the assessment of actual human exposure levels and quantitative health risks. Although various quantitative approaches have been explored to assess the risks from foodborne pathogens (Membré & Guillou, 2016) and antimicrobial resistance (Caffrey et al., 2019), systematic assessment frameworks (particularly for viable antimicrobial-resistant human pathogens) and corresponding consumption standards remain underdeveloped. Future research should prioritize the development of comprehensive evaluation frameworks to establish actionable consumption standards grounded in dose-response and epidemiological data. For example, efforts should focus on assessing colonization potential and infectious thresholds via foodborne exposure and

characterizing the epidemiological patterns of marine-derived MRSA strains.

Bioinformatics analyses conducted at the contig and MAG levels revealed significant limitations in the currently available assembly tools, which often result in the loss or disintegration of ARGs and MGEs during assembly and binning. As a result, we observed that ARG transmission among bacteria in human-impacted coastal waters was undetectable at the MAG level using MetaCHIP and that ARG pathogenic hosts in cultured fishes remained unidentified at the contig level. Such restrictions may lead to an underestimation of ARG mobility and pathogenicity in coastal marine environments. Improvements in the available assemblers are necessary to accurately capture the full diversity and abundance of genomic context across diverse sample matrices, addressing information loss due to current assembly errors. Furthermore, we recommend combining advanced extraction techniques with whole-genome sequencing to produce long-read data for assembly, thereby reducing assembly complexity and minimizing error rates in environmental genomics studies.

Although ARG transfer was found to play an important role in shaping the resistance of human pathogens in cultured fishes, it may have a limited effect on the accumulation of typical ARGs, as revealed by no overlaps between the enriched ARGs and mobile ARGs. This suggests that the enrichment of these ARGs may be driven by the invasion and proliferation of their hosts in cultured fish originating from environmental or feed sources. However, we did not detect these ARG hosts in the putative sources, which

may be due to information loss during metagenomic assembly. Therefore, beyond metagenomic analysis, culture-based experiments focusing on the enrichment of these ARG hosts in cultured fishes and putative sources, as well as their potential dissemination between these compartments, are essential to elucidate the underlying mechanisms and resultant ARG accumulation.

Antibiotics are commonly used in mariculture to treat fish infections through immersion therapies, often at high concentrations reaching ppm levels. These concentrations exert considerable selective pressures, promoting the development and spread of antimicrobial resistance in mariculture environments and increasing the potential for transmission to human pathogens. This creates new challenges for both seafood safety and the sustainable management of mariculture systems. Therefore, we recommend comprehensive investigations to assess the impacts of antibiotic exposure on shifts in bacterial communities and the emergence of antimicrobial resistance and resistant pathogens in farming systems and cultured fishes. Such research is critical to inform effective guidelines for fish disease treatment and pollution control in mariculture, with the goal of safeguarding human health.

Finally, although our research underscores the urgency of intervention, the implementation of sustainable wastewater discharge and mariculture reforms remains hindered by several obstacles, including technical constraints, economic barriers, and regulatory gaps.

1. Technical constraints

- Current water quality monitoring focuses mainly on chemical indicators in wastewater, seawater, and mariculture systems, with limited tracking of antimicrobial resistance (AMR) and antibiotic-resistant bacteria (ARB).
- Detecting pathogens and ARB remains slow and costly, hindering effective surveillance. There is an urgent need for rapid, affordable AMR detection methods to improve monitoring and response.

2. Economic barriers

- Upgrading wastewater treatment and aquaculture practices (e.g., feeding patterns) requires substantial investment in research and infrastructure.
- While reforms offer long-term public health benefits, businesses and governments often prioritize short-term financial concerns.
- If consumers are unwilling to pay a premium for "safer" seafood, farmers have little motivation to adopt stricter practices.

3. Regulatory gaps

- Remote mariculture operations make enforcement difficult
- Existing effluent standards do not explicitly regulate ARB

To remove these barriers, cost-benefit analysis (CBA) can serve as a powerful tool. In recent years, it has been applied to optimize existing aquaculture practices for sustainable development (Samat et al., 2024). In this context, this approach can be used to reform feeding patterns (for example, replacing dry pellets and trash fish with other baits free of ARG and ARB contamination) to achieve environmentally friendly and

seafood-safe feeding practices, thereby improving seafood safety and increasing its value. Additionally, CBA has demonstrated success in mitigating microbial risks in drinking water (Bergion et al., 2018) and in elevating wastewater treatment standards (Lavee, 2011). Building on these achievements, Hong Kong can harness CBA to guide technological and policy innovations, strengthening ARG and ARB monitoring in seawater and wastewater. These measures can improve coastal water quality, support recreational activities, and safeguard the health of coastal communities, ultimately fostering a more sustainable and resilient marine ecosystem. Future research should incorporate cost–benefit analyses to guide effective implementation.

Appendix

1. Human-impacted bacterial species retrieved from the comparable study of top 5000 bacteria (accounting for over 95% of the total relative abundance in each sample) between human-impacted coastal waters and pristine ocean systems (LEfSe, LDA score > 2, $p < 0.05$).

Species	Phylum	Log10 average abundance	Enriched group	LDA	KW p-value
Planktomarina temperata	*Proteobacteria*	4.67	Coastal	4.40	0.00
Candidatus Pelagibacter sp. HIMB1321	*Proteobacteria*	4.46	Coastal	3.56	0.01
Aequoribacter fuscus	*Proteobacteria*	4.28	Coastal	3.96	0.00
Colwellia sp. PAMC 20917	*Proteobacteria*	4.04	Coastal	3.72	0.00
Escherichia coli	*Proteobacteria*	3.77	Coastal	3.29	0.00
Marivivens sp. JLT3646	*Proteobacteria*	3.82	Coastal	3.48	0.00
Phaeobacter gallaeciensis	*Proteobacteria*	3.78	Coastal	3.45	0.00
Salmonella enterica	*Proteobacteria*	3.67	Coastal	3.16	0.00
Aestuarium zhoushanense	*Proteobacteria*	3.74	Coastal	3.39	0.00
Pseudoalteromonas shioyasakiensis	*Proteobacteria*	3.70	Coastal	3.31	0.00
Klebsiella pneumoniae	*Proteobacteria*	3.68	Coastal	3.27	0.00
Pseudoalteromonas arctica	*Proteobacteria*	3.67	Coastal	3.40	0.00
Pseudomonas sp. CIP-10	*Proteobacteria*	3.64	Coastal	3.29	0.00
Pseudoalteromonas agarivorans	*Proteobacteria*	3.58	Coastal	3.08	0.00
Phaeobacter inhibens	*Proteobacteria*	3.63	Coastal	3.30	0.00
Sulfitobacter pseudonitzschiae	*Proteobacteria*	3.54	Coastal	3.22	0.00
Marinobacterium sp. LSUCC0821	*Proteobacteria*	3.53	Coastal	3.20	0.00
Arcobacter aquimarinus	*Proteobacteria*	3.51	Coastal	3.18	0.00
Sulfitobacter mediterraneus	*Proteobacteria*	3.49	Coastal	3.17	0.00
Aliarcobacter cryaerophilus	*Proteobacteria*	3.48	Coastal	3.12	0.00
Pseudoalteromonas sp. PS1M3	*Proteobacteria*	3.43	Coastal	3.05	0.00
Pseudoalteromonas marina	*Proteobacteria*	3.41	Coastal	2.98	0.00
Vibrio chagasii	*Proteobacteria*	3.42	Coastal	3.17	0.00
Vibrio cholerae	*Proteobacteria*	3.40	Coastal	3.03	0.00
Yoonia vestfoldensis	*Proteobacteria*	3.41	Coastal	3.11	0.00
Acinetobacter baumannii	*Proteobacteria*	3.35	Coastal	2.85	0.00
Shewanella livingstonensis	*Proteobacteria*	3.40	Coastal	3.12	0.00
Reinekea forsetii	*Proteobacteria*	3.35	Coastal	3.03	0.00
Pseudoarcobacter acticola	*Proteobacteria*	3.34	Coastal	2.99	0.00
Shewanella sp. Arc9-LZ	*Proteobacteria*	3.34	Coastal	3.06	0.00

Malaciobacter pacificus	*Proteobacteria*	3.32	Coastal	2.97	0.00
Aliarcobacter skirrowii	*Proteobacteria*	3.31	Coastal	2.96	0.00
Shewanella sp. M2	*Proteobacteria*	3.27	Coastal	2.99	0.00
Pseudomonas aeruginosa	*Proteobacteria*	3.21	Coastal	2.72	0.00
Octadecabacter arcticus	*Proteobacteria*	3.25	Coastal	2.92	0.00
Colwellia psychrerythraea	*Proteobacteria*	3.25	Coastal	2.92	0.00
Shewanella frigidimarina	*Proteobacteria*	3.25	Coastal	2.97	0.00
Aliarcobacter butzleri	*Proteobacteria*	3.21	Coastal	2.81	0.00
Pseudoalteromonas sp. Xi13	*Proteobacteria*	3.16	Coastal	2.64	0.00
Cognatishimia activa	*Proteobacteria*	3.23	Coastal	2.91	0.00
Celeribacter marinus	*Proteobacteria*	3.22	Coastal	2.90	0.00
Yersinia pestis	*Proteobacteria*	3.20	Coastal	2.84	0.00
Tritonibacter mobilis	*Proteobacteria*	3.18	Coastal	2.75	0.00
Pseudoalteromonas sp. 13-15	*Proteobacteria*	3.17	Coastal	2.76	0.00
Pseudoalteromonas atlantica	*Proteobacteria*	3.13	Coastal	2.63	0.00
Pseudoalteromonas sp. SiA1	*Proteobacteria*	3.17	Coastal	2.77	0.00
Pseudoalteromonas undina	*Proteobacteria*	3.15	Coastal	2.73	0.00
Sulfitobacter sp. SK025	*Proteobacteria*	3.13	Coastal	2.71	0.00
Vibrio parahaemolyticus	*Proteobacteria*	3.16	Coastal	2.80	0.00
Pseudoalteromonas tetraodonis	*Proteobacteria*	3.12	Coastal	2.69	0.00
Candidatus Puniceispirillum marinum	*Proteobacteria*	3.07	Coastal	2.44	0.00
Pseudoalteromonas issachenkonii	*Proteobacteria*	3.15	Coastal	2.79	0.00
Glaciecola nitratireducens	*Proteobacteria*	3.15	Coastal	2.81	0.00
Sulfitobacter sp. JL08	*Proteobacteria*	3.15	Coastal	2.82	0.00
Pseudoalteromonas sp. SM9913	*Proteobacteria*	3.12	Coastal	2.72	0.00
Sulfitobacter sp. SK012	*Proteobacteria*	3.14	Coastal	2.82	0.00
Colwellia sp. Arc7-635	*Proteobacteria*	3.13	Coastal	2.79	0.00
Octadecabacter antarcticus	*Proteobacteria*	3.12	Coastal	2.79	0.00
Octadecabacter sp. SW4	*Proteobacteria*	3.14	Coastal	2.81	0.00
Arcobacter ellisii	*Proteobacteria*	3.12	Coastal	2.75	0.00
Sulfitobacter sp. D7	*Proteobacteria*	3.11	Coastal	2.74	0.00
Sulfitobacter sp. SK011	*Proteobacteria*	3.11	Coastal	2.79	0.00
Pseudoalteromonas sp. DL-6	*Proteobacteria*	3.06	Coastal	2.62	0.00
Pseudomonas stutzeri	*Proteobacteria*	3.06	Coastal	2.64	0.00
Vibrio alginolyticus	*Proteobacteria*	3.07	Coastal	2.71	0.00
Phaeobacter piscinae	*Proteobacteria*	3.08	Coastal	2.75	0.00
Octadecabacter temperatus	*Proteobacteria*	3.09	Coastal	2.77	0.00
Colwellia sp. 20A7	*Proteobacteria*	3.07	Coastal	2.74	0.00
Ruegeria sp. SCSIO 43209	*Proteobacteria*	3.08	Coastal	2.75	0.00
Celeribacter baekdonensis	*Proteobacteria*	3.07	Coastal	2.74	0.00
Pseudoalteromonas piratica	*Proteobacteria*	3.07	Coastal	2.73	0.00
Vibrio sp. THAF190c	*Proteobacteria*	3.07	Coastal	2.82	0.00
Ruegeria sp. THAF33	*Proteobacteria*	3.05	Coastal	2.72	0.00

Photobacterium damselae	*Proteobacteria*	2.97	Coastal	2.41	0.00
Sulfitobacter sp. BSw21498	*Proteobacteria*	3.04	Coastal	2.72	0.00
Arcobacter cloacae	*Proteobacteria*	3.02	Coastal	2.64	0.00
Vibrio navarrensis	*Proteobacteria*	3.03	Coastal	2.70	0.00
Halocynthiibacter arcticus	*Proteobacteria*	3.01	Coastal	2.67	0.00
Thalassobius gelatinovorus	*Proteobacteria*	3.02	Coastal	2.69	0.00
Halomonas sp. JS92-SW72	*Proteobacteria*	2.98	Coastal	2.58	0.00
Sulfitobacter alexandrii	*Proteobacteria*	3.01	Coastal	2.67	0.00
Thalassolituus oleivorans	*Proteobacteria*	2.99	Coastal	2.58	0.00
Sulfitobacter sp. THAF37	*Proteobacteria*	3.00	Coastal	2.67	0.00
Vibrio cyclitrophicus	*Proteobacteria*	2.92	Coastal	2.44	0.00
Roseovarius indicus	*Proteobacteria*	2.94	Coastal	2.44	0.00
Ruegeria pomeroyi	*Proteobacteria*	2.99	Coastal	2.65	0.00
Vibrio splendidus	*Proteobacteria*	2.96	Coastal	2.61	0.00
Arcobacter defluvii	*Proteobacteria*	2.97	Coastal	2.57	0.00
Roseobacter litoralis	*Proteobacteria*	2.98	Coastal	2.65	0.00
Roseobacter denitrificans	*Proteobacteria*	2.98	Coastal	2.65	0.00
Marinovum algicola	*Proteobacteria*	2.98	Coastal	2.63	0.00
Pseudohalocynthiibacter aestuariivivens	*Proteobacteria*	2.97	Coastal	2.63	0.00
Shewanella inventionis	*Proteobacteria*	2.96	Coastal	2.66	0.00
Roseovarius faecimaris	*Proteobacteria*	2.96	Coastal	2.63	0.00
Pseudoalteromonas aliena	*Proteobacteria*	2.95	Coastal	2.62	0.00
Profundibacter amoris	*Proteobacteria*	2.96	Coastal	2.62	0.00
Pseudoalteromonas sp. LC2018020214	*Proteobacteria*	2.95	Coastal	2.66	0.00
Vibrio kanaloae	*Proteobacteria*	2.96	Coastal	2.65	0.00
Paraglaciecola psychrophila	*Proteobacteria*	2.94	Coastal	2.57	0.00
Pseudorhodobacter turbinis	*Proteobacteria*	2.95	Coastal	2.61	0.00
Klebsiella michiganensis	*Proteobacteria*	2.95	Coastal	2.60	0.00
Poseidonibacter lekithochrous	*Proteobacteria*	2.91	Coastal	2.44	0.00
Citrobacter freundii	*Proteobacteria*	2.92	Coastal	2.52	0.00
Vibrio natriegens	*Proteobacteria*	2.95	Coastal	2.62	0.00
Parasedimentitalea marina	*Proteobacteria*	2.94	Coastal	2.59	0.00
Pseudoalteromonas sp. 3J6	*Proteobacteria*	2.87	Coastal	2.36	0.00
Pseudoalteromonas sp. 16-SW-7	*Proteobacteria*	2.93	Coastal	2.62	0.00
Arcobacter venerupis	*Proteobacteria*	2.91	Coastal	2.51	0.00
Shewanella glacialimarina	*Proteobacteria*	2.93	Coastal	2.63	0.00
Sulfitobacter sp. JK7-1	*Proteobacteria*	2.93	Coastal	2.61	0.00
Actibacterium sp. EMB200-NS6	*Proteobacteria*	2.91	Coastal	2.53	0.00
Antarctobacter heliothermus	*Proteobacteria*	2.93	Coastal	2.60	0.00
Aliarcobacter cibarius	*Proteobacteria*	2.89	Coastal	2.46	0.00
Halarcobacter bivalviorum	*Proteobacteria*	2.89	Coastal	2.47	0.01
Phaeobacter porticola	*Proteobacteria*	2.92	Coastal	2.58	0.00

Pseudoalteromonas prydzensis	*Proteobacteria*	2.90	Coastal	2.53	0.00
Arcobacter suis	*Proteobacteria*	2.89	Coastal	2.51	0.00
Ruegeria sp. AD91A	*Proteobacteria*	2.91	Coastal	2.58	0.00
Pseudomonas putida	*Proteobacteria*	2.86	Coastal	2.41	0.00
Tateyamaria omphalii	*Proteobacteria*	2.90	Coastal	2.57	0.00
Salipiger abyssi	*Proteobacteria*	2.89	Coastal	2.54	0.00
Acinetobacter johnsonii	*Proteobacteria*	2.87	Coastal	2.46	0.00
Colwellia sp. MT41	*Proteobacteria*	2.88	Coastal	2.54	0.00
Vibrio sp. Scap24	*Proteobacteria*	2.90	Coastal	2.57	0.00
Vibrio fluvialis	*Proteobacteria*	2.89	Coastal	2.55	0.00
Celeribacter ethanolicus	*Proteobacteria*	2.89	Coastal	2.55	0.00
Leisingera methylohalidivorans	*Proteobacteria*	2.89	Coastal	2.56	0.00
Colwellia sp. Arc7-D	*Proteobacteria*	2.87	Coastal	2.52	0.00
Enterobacter hormaechei	*Proteobacteria*	2.83	Coastal	2.37	0.00
Aeromonas caviae	*Proteobacteria*	2.87	Coastal	2.53	0.00
Cognaticolwellia beringensis	*Proteobacteria*	2.85	Coastal	2.50	0.00
Roseovarius sp. TM1035	*Proteobacteria*	2.86	Coastal	2.51	0.00
Ruegeria sp. TM1040	*Proteobacteria*	2.86	Coastal	2.53	0.00
Roseobacter ponti	*Proteobacteria*	2.84	Coastal	2.51	0.00
Roseovarius mucosus	*Proteobacteria*	2.83	Coastal	2.47	0.00
Leisingera sp. NJS201	*Proteobacteria*	2.83	Coastal	2.50	0.00
Leisingera aquaemixtae	*Proteobacteria*	2.84	Coastal	2.49	0.00
Dinoroseobacter shibae	*Proteobacteria*	2.83	Coastal	2.48	0.00
Mameliella alba	*Proteobacteria*	2.83	Coastal	2.49	0.00
Thalassococcus sp. S3	*Proteobacteria*	2.83	Coastal	2.50	0.00
Sulfitobacter pontiacus	*Proteobacteria*	2.80	Coastal	2.40	0.00
Vibrio ziniensis	*Proteobacteria*	2.83	Coastal	2.48	0.00
Vibrio crassostreae	*Proteobacteria*	2.82	Coastal	2.49	0.00
Shewanella polaris	*Proteobacteria*	2.82	Coastal	2.53	0.00
Shewanella baltica	*Proteobacteria*	2.80	Coastal	2.46	0.00
Pseudoalteromonas translucida	*Proteobacteria*	2.80	Coastal	2.46	0.00
Colwellia sp. PAMC 21821	*Proteobacteria*	2.79	Coastal	2.44	0.00
Pseudoalteromonas paragorgicola	*Proteobacteria*	2.80	Coastal	2.51	0.00
Shewanella putrefaciens	*Proteobacteria*	2.77	Coastal	2.37	0.00
Boseongicola sp. CCM32	*Proteobacteria*	2.79	Coastal	2.44	0.00
Sagittula sp. P11	*Proteobacteria*	2.76	Coastal	2.35	0.00
Vibrio vulnificus	*Proteobacteria*	2.77	Coastal	2.37	0.00
Pukyongiella litopenaei	*Proteobacteria*	2.78	Coastal	2.43	0.00
Congregibacter litoralis	*Proteobacteria*	2.76	Coastal	2.35	0.00
Sulfitobacter sp. B30-2	*Proteobacteria*	2.76	Coastal	2.35	0.00
Moritella marina	*Proteobacteria*	2.77	Coastal	2.42	0.00
Arcobacter nitrofigilis	*Proteobacteria*	2.73	Coastal	2.26	0.05
Bermanella marisrubri	*Proteobacteria*	2.76	Coastal	2.40	0.00
Pseudoalteromonas arabiensis	*Proteobacteria*	2.75	Coastal	2.34	0.00

Pseudoalteromonas spongiae	*Proteobacteria*	2.76	Coastal	2.37	0.00
Vibrio anguillarum	*Proteobacteria*	2.75	Coastal	2.39	0.00
Pseudomonas fluorescens	*Proteobacteria*	2.72	Coastal	2.31	0.00
Vibrio campbellii	*Proteobacteria*	2.73	Coastal	2.36	0.00
Leisingera sp. NJS204	*Proteobacteria*	2.75	Coastal	2.42	0.00
Roseovarius sp. THAF9	*Proteobacteria*	2.74	Coastal	2.39	0.00
Shewanella sp. MEBiC00475	*Proteobacteria*	2.74	Coastal	2.45	0.00
Paracoccus yeei	*Proteobacteria*	2.73	Coastal	2.35	0.00
Proteus mirabilis	*Proteobacteria*	2.68	Coastal	2.17	0.00
Pseudoalteromonas donghaensis	*Proteobacteria*	2.73	Coastal	2.36	0.00
Pseudoalteromonas carrageenovora	*Proteobacteria*	2.69	Coastal	2.23	0.00
Litorilituus sediminis	*Proteobacteria*	2.71	Coastal	2.34	0.00
Celeribacter indicus	*Proteobacteria*	2.72	Coastal	2.36	0.00
Cereibacter sphaeroides	*Proteobacteria*	2.71	Coastal	2.32	0.00
Pacificitalea manganoxidans	*Proteobacteria*	2.71	Coastal	2.35	0.00
Rhodobaca barguzinensis	*Proteobacteria*	2.72	Coastal	2.38	0.00
Klebsiella quasipneumoniae	*Proteobacteria*	2.64	Coastal	2.10	0.00
Jannaschia sp. CCS1	*Proteobacteria*	2.70	Coastal	2.37	0.00
Psychrosphaera aestuarii	*Proteobacteria*	2.68	Coastal	2.27	0.00
Vibrio mimicus	*Proteobacteria*	2.69	Coastal	2.33	0.00
Burkholderia pseudomallei	*Proteobacteria*	2.62	Coastal	2.03	0.00
Pseudoalteromonas phenolica	*Proteobacteria*	2.68	Coastal	2.30	0.00
Vibrio cidicii	*Proteobacteria*	2.69	Coastal	2.32	0.00
Moraxella osloensis	*Proteobacteria*	2.67	Coastal	2.26	0.00
Pseudopuniceibacterium antarcticum	*Proteobacteria*	2.69	Coastal	2.36	0.00
Roseibacterium elongatum	*Proteobacteria*	2.69	Coastal	2.35	0.00
Altererythrobacter ishigakiensis	*Proteobacteria*	2.69	Coastal	2.37	0.00
Pannonibacter phragmitetus	*Proteobacteria*	2.61	Coastal	2.05	0.00
Aeromonas media	*Proteobacteria*	2.68	Coastal	2.37	0.00
Thalassotalea sp. LPB0316	*Proteobacteria*	2.67	Coastal	2.30	0.00
Rhodobacter sp. LPB0142	*Proteobacteria*	2.68	Coastal	2.33	0.00
Oceanicola sp. D3	*Proteobacteria*	2.67	Coastal	2.31	0.00
Agrobacterium tumefaciens	*Proteobacteria*	2.64	Coastal	2.23	0.00
Qingshengfaniella alkalisoli	*Proteobacteria*	2.67	Coastal	2.33	0.00
Brevirhabdus pacifica	*Proteobacteria*	2.66	Coastal	2.30	0.00
Stenotrophomonas maltophilia	*Proteobacteria*	2.63	Coastal	2.20	0.00
Pseudoalteromonas nigrifaciens	*Proteobacteria*	2.66	Coastal	2.34	0.00
Haemophilus influenzae	*Proteobacteria*	2.59	Coastal	2.01	0.00
Salipiger sp. CCB-MM3	*Proteobacteria*	2.66	Coastal	2.30	0.00
Sorangium cellulosum	*Proteobacteria*	2.59	Coastal	2.04	0.00
Pseudoalteromonas sp. Bsw20308	*Proteobacteria*	2.66	Coastal	2.36	0.00

Pseudoalteromonas sp. 1_2015MBL_MicDiv	*Proteobacteria*	2.64	Coastal	2.33	0.00
Enterobacter cloacae	*Proteobacteria*	2.62	Coastal	2.21	0.00
Roseicitreum antarcticum	*Proteobacteria*	2.63	Coastal	2.29	0.00
Alteromonas sp. MB-3u-76	*Proteobacteria*	2.56	Coastal	2.01	0.00
Pseudoalteromonas luteoviolacea	*Proteobacteria*	2.58	Coastal	2.10	0.00
Rhodobacter capsulatus	*Proteobacteria*	2.62	Coastal	2.27	0.00
Rhizobium leguminosarum	*Proteobacteria*	2.58	Coastal	2.11	0.00
Shewanella aestuarii	*Proteobacteria*	2.61	Coastal	2.25	0.00
Frigidibacter mobilis	*Proteobacteria*	2.62	Coastal	2.26	0.00
Gemmobacter aquarius	*Proteobacteria*	2.63	Coastal	2.29	0.00
Pseudoalteromonas espejiana	*Proteobacteria*	2.57	Coastal	2.09	0.00
Ketogulonicigenium vulgare	*Proteobacteria*	2.62	Coastal	2.29	0.00
Aeromonas veronii	*Proteobacteria*	2.61	Coastal	2.24	0.00
Roseovarius sp. THAF8	*Proteobacteria*	2.61	Coastal	2.27	0.00
Paraglaciecola sp. L3A3	*Proteobacteria*	2.58	Coastal	2.15	0.00
Limnohabitans sp. 63ED37-2	*Proteobacteria*	2.62	Coastal	2.32	0.00
Venatorbacter cucullus	*Proteobacteria*	2.58	Coastal	2.19	0.00
Pseudooceanicola algae	*Proteobacteria*	2.59	Coastal	2.25	0.00
Roseivivax sp. THAF30	*Proteobacteria*	2.59	Coastal	2.24	0.00
Aliivibrio fischeri	*Proteobacteria*	2.55	Coastal	2.10	0.00
Roseovarius sp. SCSIO 43702	*Proteobacteria*	2.57	Coastal	2.20	0.00
Vibrio bathopelagicus	*Proteobacteria*	2.55	Coastal	2.17	0.00
Alteromonas stellipolaris	*Proteobacteria*	2.52	Coastal	2.02	0.00
Vibrio azureus	*Proteobacteria*	2.56	Coastal	2.19	0.00
Morganella morganii	*Proteobacteria*	2.54	Coastal	2.10	0.00
Oceanicoccus sagamiensis	*Proteobacteria*	2.55	Coastal	2.15	0.00
Paracoccus sp. BM15	*Proteobacteria*	2.57	Coastal	2.23	0.00
Paracoccus kondratievae	*Proteobacteria*	2.56	Coastal	2.22	0.00
Pseudoalteromonas lipolytica	*Proteobacteria*	2.56	Coastal	2.21	0.00
Psychromonas ingrahamii	*Proteobacteria*	2.51	Coastal	2.08	0.00
Rhodovulum sulfidophilum	*Proteobacteria*	2.54	Coastal	2.16	0.00
Acinetobacter towneri	*Proteobacteria*	2.54	Coastal	2.15	0.00
Jannaschia sp. J12C1-MA-4	*Proteobacteria*	2.55	Coastal	2.20	0.00
Rhodovulum sp. P5	*Proteobacteria*	2.54	Coastal	2.19	0.00
Cycloclasticus sp. PY97N	*Proteobacteria*	2.51	Coastal	2.06	0.00
Arcobacter anaerophilus	*Proteobacteria*	2.50	Coastal	2.01	0.00
Halioglobus japonicus	*Proteobacteria*	2.51	Coastal	2.10	0.00
Acidovorax sp. KKS102	*Proteobacteria*	2.53	Coastal	2.17	0.00
Roseovarius sp. THAF27	*Proteobacteria*	2.53	Coastal	2.18	0.00
Roseivivax sp. THAF197b	*Proteobacteria*	2.53	Coastal	2.18	0.00
Tabrizicola piscis	*Proteobacteria*	2.53	Coastal	2.19	0.00
Pseudoalteromonas ulvae	*Proteobacteria*	2.50	Coastal	2.08	0.00
Maribius sp. THAF1	*Proteobacteria*	2.53	Coastal	2.20	0.00

Maritalea myrionectae	*Proteobacteria*	2.50	Coastal	2.10	0.00
Marinomonas primoryensis	*Proteobacteria*	2.49	Coastal	2.07	0.00
Vibrio diabolicus	*Proteobacteria*	2.52	Coastal	2.18	0.00
Ketogulonicigenium robustum	*Proteobacteria*	2.52	Coastal	2.18	0.00
Acinetobacter indicus	*Proteobacteria*	2.48	Coastal	2.03	0.00
Rhizobium pusense	*Proteobacteria*	2.47	Coastal	2.01	0.00
Salipiger pacificus	*Proteobacteria*	2.51	Coastal	2.14	0.00
Vibrio atlanticus	*Proteobacteria*	2.49	Coastal	2.11	0.00
Halioglobus pacificus	*Proteobacteria*	2.49	Coastal	2.09	0.00
Thalassotalea crassostreae	*Proteobacteria*	2.47	Coastal	2.05	0.00
Pseudomonas syringae	*Proteobacteria*	2.47	Coastal	2.04	0.00
Aeromonas salmonicida	*Proteobacteria*	2.50	Coastal	2.16	0.00
Acinetobacter venetianus	*Proteobacteria*	2.48	Coastal	2.08	0.00
Monaibacterium sp. ALG8	*Proteobacteria*	2.48	Coastal	2.10	0.00
Acinetobacter lwoffii	*Proteobacteria*	2.45	Coastal	2.02	0.00
Aeromonas hydrophila	*Proteobacteria*	2.46	Coastal	2.09	0.00
Paracoccus zhejiangensis	*Proteobacteria*	2.46	Coastal	2.08	0.00
Halioglobus maricola	*Proteobacteria*	2.45	Coastal	2.06	0.00
Haematobacter massiliensis	*Proteobacteria*	2.47	Coastal	2.11	0.00
Vibrio owensii	*Proteobacteria*	2.47	Coastal	2.12	0.00
Hyphomonas sp. Mor2	*Proteobacteria*	2.47	Coastal	2.15	0.00
Paracoccus sp. H4-D09	*Proteobacteria*	2.47	Coastal	2.13	0.00
Vibrio mediterranei	*Proteobacteria*	2.45	Coastal	2.05	0.00
Shewanella sp. Scap07	*Proteobacteria*	2.45	Coastal	2.09	0.00
Moritella sp. 24	*Proteobacteria*	2.44	Coastal	2.05	0.00
Cycloclasticus sp. P1	*Proteobacteria*	2.44	Coastal	2.01	0.00
Vibrio sp. dhg	*Proteobacteria*	2.46	Coastal	2.14	0.00
Psychrosphaera sp. MTZ26	*Proteobacteria*	2.43	Coastal	2.02	0.00
Marinobacter sp. BSs20148	*Proteobacteria*	2.45	Coastal	2.12	0.00
Phaeobacter sp. LSS9	*Proteobacteria*	2.43	Coastal	2.06	0.00
Fuscovulum blasticum	*Proteobacteria*	2.44	Coastal	2.09	0.00
Thioclava nitratireducens	*Proteobacteria*	2.42	Coastal	2.01	0.00
Vibrio scophthalmi	*Proteobacteria*	2.42	Coastal	2.05	0.00
Vibrio harveyi	*Proteobacteria*	2.42	Coastal	2.07	0.00
Diaphorobacter sp. JS3051	*Proteobacteria*	2.43	Coastal	2.12	0.00
Pseudoalteromonas sp. JSTW	*Proteobacteria*	2.41	Coastal	2.03	0.00
Paracoccus aminophilus	*Proteobacteria*	2.42	Coastal	2.07	0.00
Shewanella japonica	*Proteobacteria*	2.41	Coastal	2.05	0.00
Labrenzia sp. PHM005	*Proteobacteria*	2.40	Coastal	2.03	0.00
Bacterioplanes sanyensis	*Proteobacteria*	2.40	Coastal	2.01	0.00
Xanthomonas citri	*Proteobacteria*	2.41	Coastal	2.05	0.00
Shewanella sp. WPAGA9	*Proteobacteria*	2.40	Coastal	2.04	0.00
Litoricola lipolytica	*Proteobacteria*	2.40	Coastal	2.07	0.00
Rhodobacter sp. N10	*Proteobacteria*	2.40	Coastal	2.04	0.00

Paracoccus pantotrophus	*Proteobacteria*	2.39	Coastal	2.03	0.00
Roseivivax sp. THAF40	*Proteobacteria*	2.37	Coastal	2.03	0.00
Vibrio rotiferianus	*Proteobacteria*	2.37	Coastal	2.02	0.00
Tolumonas auensis	*Proteobacteria*	2.34	Coastal	2.00	0.00
Polynucleobacter sp. AM-7D1	*Proteobacteria*	2.31	Coastal	2.01	0.00
Bacillus cereus	*Firmicutes*	3.36	Coastal	2.83	0.00
Faecalibacterium prausnitzii	*Firmicutes*	3.26	Coastal	2.95	0.00
Staphylococcus aureus	*Firmicutes*	3.03	Coastal	2.04	0.01
Megamonas funiformis	*Firmicutes*	2.97	Coastal	2.61	0.00
Anaerostipes hadrus	*Firmicutes*	2.69	Coastal	2.31	0.00
Roseburia intestinalis	*Firmicutes*	2.66	Coastal	2.32	0.00
Bacillus anthracis	*Firmicutes*	2.62	Coastal	2.17	0.00
[Ruminococcus] gnavus	*Firmicutes*	2.64	Coastal	2.30	0.00
Anaerobutyricum hallii	*Firmicutes*	2.60	Coastal	2.20	0.00
[Ruminococcus] torques	*Firmicutes*	2.57	Coastal	2.20	0.00
Lachnospira eligens	*Firmicutes*	2.52	Coastal	2.15	0.00
Blautia sp. SC05B48	*Firmicutes*	2.50	Coastal	2.16	0.00
Ruminococcus bicirculans	*Firmicutes*	2.43	Coastal	2.06	0.00
Blautia massiliensis	*Firmicutes*	2.43	Coastal	2.10	0.00
Blautia obeum	*Firmicutes*	2.40	Coastal	2.05	0.00
Synechococcus sp. LTW-R	*Cyanobacteria*	4.08	Coastal	3.73	0.03
Synechococcus sp. Minos11	*Cyanobacteria*	3.61	Coastal	3.00	0.02
Synechococcus sp. ROS8604	*Cyanobacteria*	3.35	Coastal	2.96	0.01
Thermostichus vulcanus	*Cyanobacteria*	2.59	Coastal	2.18	0.00
Geminocystis sp. NIES-3709	*Cyanobacteria*	2.50	Coastal	2.00	0.00
Thermosynechococcus vestitus	*Cyanobacteria*	2.48	Coastal	2.05	0.00
Formosa sp. Hel3_A1_48	*Bacteroidetes*	3.85	Coastal	3.44	0.00
Formosa sp. Hel1_33_131	*Bacteroidetes*	3.88	Coastal	3.56	0.00
Flavobacterium sp. Sr18	*Bacteroidetes*	3.61	Coastal	3.34	0.00
Phocaeicola vulgatus	*Bacteroidetes*	3.41	Coastal	3.10	0.00
Mesoflavibacter sp. SCSIO 43206	*Bacteroidetes*	3.29	Coastal	2.88	0.00
Salegentibacter mishustinae	*Bacteroidetes*	3.25	Coastal	2.77	0.00
Prevotella copri	*Bacteroidetes*	3.30	Coastal	3.01	0.00
Polaribacter sp. Hel1_33_78	*Bacteroidetes*	3.22	Coastal	2.74	0.00
Polaribacter sp. L12M9	*Bacteroidetes*	3.18	Coastal	2.67	0.00
Polaribacter sp. SA4-10	*Bacteroidetes*	3.16	Coastal	2.62	0.00
Polaribacter sp. MED152	*Bacteroidetes*	3.17	Coastal	2.70	0.00
Urechidicola croceus	*Bacteroidetes*	3.13	Coastal	2.55	0.00
Polaribacter sp. SA4-12	*Bacteroidetes*	3.13	Coastal	2.63	0.00
Tenacibaculum sp. SZ-18	*Bacteroidetes*	3.13	Coastal	2.68	0.00
Polaribacter sp. KT25b	*Bacteroidetes*	3.11	Coastal	2.60	0.00
Polaribacter sp. ALD11	*Bacteroidetes*	3.06	Coastal	2.45	0.00
Polaribacter litorisediminis	*Bacteroidetes*	3.11	Coastal	2.66	0.00
Polaribacter vadi	*Bacteroidetes*	3.11	Coastal	2.63	0.00

Polaribacter reichenbachii	*Bacteroidetes*	3.10	Coastal	2.62	0.00
Winogradskyella sp. PG-2	*Bacteroidetes*	3.11	Coastal	2.66	0.00
Flavobacterium sp. F-29	*Bacteroidetes*	3.10	Coastal	2.78	0.00
Polaribacter haliotis	*Bacteroidetes*	3.05	Coastal	2.54	0.00
Tenacibaculum todarodis	*Bacteroidetes*	3.05	Coastal	2.58	0.00
Flavobacterium sp. I3-2	*Bacteroidetes*	3.01	Coastal	2.39	0.00
Psychroserpens sp. NJDZ02	*Bacteroidetes*	3.02	Coastal	2.47	0.00
Winogradskyella sp. HaHa_3_26	*Bacteroidetes*	3.03	Coastal	2.52	0.00
Oceanihabitans sp. IOP_32	*Bacteroidetes*	3.06	Coastal	2.65	0.00
Winogradskyella helgolandensis	*Bacteroidetes*	3.04	Coastal	2.61	0.00
Algibacter alginicilyticus	*Bacteroidetes*	3.03	Coastal	2.56	0.00
Flavobacterium haoranii	*Bacteroidetes*	3.03	Coastal	2.56	0.00
Flavobacterium sp. F-340	*Bacteroidetes*	3.04	Coastal	2.68	0.00
Polaribacter sp. NJDZ03	*Bacteroidetes*	3.01	Coastal	2.51	0.00
Seonamhaeicola sp. S2-3	*Bacteroidetes*	3.02	Coastal	2.58	0.00
Algibacter sp. L1A34	*Bacteroidetes*	3.02	Coastal	2.61	0.00
Polaribacter sp. L3A8	*Bacteroidetes*	3.01	Coastal	2.51	0.00
Tenacibaculum jejuense	*Bacteroidetes*	3.01	Coastal	2.52	0.00
Winogradskyella sp. PC-19	*Bacteroidetes*	3.00	Coastal	2.51	0.00
Cellulophaga lytica	*Bacteroidetes*	2.99	Coastal	2.48	0.00
Olleya aquimaris	*Bacteroidetes*	2.98	Coastal	2.39	0.00
Polaribacter sp. G4M1	*Bacteroidetes*	3.01	Coastal	2.52	0.00
Bacteroides uniformis	*Bacteroidetes*	3.06	Coastal	2.74	0.00
Lacinutrix sp. 5H-3-7-4	*Bacteroidetes*	2.98	Coastal	2.47	0.00
Winogradskyella forsetii	*Bacteroidetes*	3.00	Coastal	2.57	0.00
Algibacter sp. L3A6	*Bacteroidetes*	3.00	Coastal	2.58	0.00
Zunongwangia profunda	*Bacteroidetes*	2.92	Coastal	2.13	0.00
Siansivirga zeaxanthinifaciens	*Bacteroidetes*	2.99	Coastal	2.58	0.00
Polaribacter sp. KT 15	*Bacteroidetes*	2.96	Coastal	2.44	0.00
Polaribacter sp. SM13	*Bacteroidetes*	2.96	Coastal	2.44	0.00
Polaribacter sp. Q13	*Bacteroidetes*	2.96	Coastal	2.44	0.00
Flavobacterium columnare	*Bacteroidetes*	2.94	Coastal	2.38	0.00
Tenacibaculum maritimum	*Bacteroidetes*	2.95	Coastal	2.46	0.00
Tenacibaculum dicentrarchi	*Bacteroidetes*	2.96	Coastal	2.51	0.00
Winogradskyella sediminis	*Bacteroidetes*	2.96	Coastal	2.50	0.00
Lacinutrix sp. Bg11-31	*Bacteroidetes*	2.94	Coastal	2.47	0.00
Lacinutrix sp. WUR7	*Bacteroidetes*	2.95	Coastal	2.49	0.00
Changchengzhania lutea	*Bacteroidetes*	2.96	Coastal	2.57	0.00
Polaribacter sp. BM10	*Bacteroidetes*	2.93	Coastal	2.40	0.00
Flavobacterium sediminis	*Bacteroidetes*	2.93	Coastal	2.41	0.00
Winogradskyella schleiferi	*Bacteroidetes*	2.95	Coastal	2.52	0.00
Zunongwangia sp. SCSIO 43204	*Bacteroidetes*	2.90	Coastal	2.27	0.00
Lutibacter profundi	*Bacteroidetes*	2.90	Coastal	2.30	0.00
Salegentibacter sp. T436	*Bacteroidetes*	2.92	Coastal	2.44	0.00

Lacinutrix venerupis	*Bacteroidetes*	2.91	Coastal	2.39	0.00
Flavobacterium sp. KK2020170	*Bacteroidetes*	2.91	Coastal	2.41	0.00
Mesoflavibacter profundi	*Bacteroidetes*	2.91	Coastal	2.43	0.00
Phocaeicola dorei	*Bacteroidetes*	2.97	Coastal	2.65	0.00
Flavivirga eckloniae	*Bacteroidetes*	2.93	Coastal	2.51	0.00
Tenacibaculum mesophilum	*Bacteroidetes*	2.92	Coastal	2.48	0.00
Tenacibaculum singaporense	*Bacteroidetes*	2.93	Coastal	2.50	0.00
Polaribacter sp. HaHaR_3_91	*Bacteroidetes*	2.90	Coastal	2.35	0.00
Flavobacterium sp. M31R6	*Bacteroidetes*	2.93	Coastal	2.58	0.00
Formosa sp. Hel1_31_208	*Bacteroidetes*	2.90	Coastal	2.45	0.00
Cloacibacterium caeni	*Bacteroidetes*	2.91	Coastal	2.46	0.00
Mariniflexile sp. TRM1-10	*Bacteroidetes*	2.91	Coastal	2.51	0.00
Marixanthomonas sp. SCSIO 43207	*Bacteroidetes*	2.88	Coastal	2.36	0.00
Winogradskyella sp. J14-2	*Bacteroidetes*	2.88	Coastal	2.35	0.00
Polaribacter sejongensis	*Bacteroidetes*	2.88	Coastal	2.37	0.00
Aquimarina sp. AD1	*Bacteroidetes*	2.86	Coastal	2.31	0.00
Maribacter cobaltidurans	*Bacteroidetes*	2.88	Coastal	2.40	0.00
Aquimarina sp. AD10	*Bacteroidetes*	2.85	Coastal	2.28	0.00
Cellulophaga sp. HaHaR_3_176	*Bacteroidetes*	2.83	Coastal	2.26	0.00
Kordia antarctica	*Bacteroidetes*	2.86	Coastal	2.39	0.00
Flavobacterium sp. 140616W15	*Bacteroidetes*	2.86	Coastal	2.44	0.00
Formosa sp. L2A11	*Bacteroidetes*	2.84	Coastal	2.34	0.00
Flavobacterium faecale	*Bacteroidetes*	2.86	Coastal	2.49	0.00
Flavobacterium sp. CECT 9288	*Bacteroidetes*	2.84	Coastal	2.41	0.00
Aquimarina sp. BL5	*Bacteroidetes*	2.83	Coastal	2.31	0.00
Flavobacterium crassostreae	*Bacteroidetes*	2.86	Coastal	2.50	0.00
Formosa sediminum	*Bacteroidetes*	2.83	Coastal	2.34	0.00
Wenyingzhuangia fucanilytica	*Bacteroidetes*	2.82	Coastal	2.29	0.00
Kordia sp. SMS9	*Bacteroidetes*	2.85	Coastal	2.43	0.00
Polaribacter sp. R2A056_3_33	*Bacteroidetes*	2.82	Coastal	2.31	0.00
Polaribacter sp. AHE13PA	*Bacteroidetes*	2.82	Coastal	2.27	0.00
Tamlana sp. s12	*Bacteroidetes*	2.83	Coastal	2.42	0.00
Flavobacterium sp. CS20	*Bacteroidetes*	2.78	Coastal	2.16	0.00
Maribacter hydrothermalis	*Bacteroidetes*	2.80	Coastal	2.27	0.00
Aequorivita sp. H23M31	*Bacteroidetes*	2.80	Coastal	2.28	0.00
Flavobacterium sp. CHNK8	*Bacteroidetes*	2.83	Coastal	2.42	0.00
Gillisia sp. Hel1_33_143	*Bacteroidetes*	2.78	Coastal	2.19	0.00
Tamlana carrageenivorans	*Bacteroidetes*	2.82	Coastal	2.41	0.00
Olleya sp. HaHaR_3_96	*Bacteroidetes*	2.79	Coastal	2.25	0.00
Cellulophaga sp. L1A9	*Bacteroidetes*	2.79	Coastal	2.25	0.00
Euzebyella marina	*Bacteroidetes*	2.81	Coastal	2.37	0.00
Formosa agariphila	*Bacteroidetes*	2.80	Coastal	2.32	0.00
Parabacteroides distasonis	*Bacteroidetes*	2.84	Coastal	2.50	0.00

Flavobacterium psychrophilum	*Bacteroidetes*	2.79	Coastal	2.35	0.00
Croceibacter atlanticus	*Bacteroidetes*	2.74	Coastal	2.05	0.00
Olleya sp. Bg11-27	*Bacteroidetes*	2.77	Coastal	2.25	0.00
Bacteroides fragilis	*Bacteroidetes*	2.82	Coastal	2.45	0.00
Flavobacterium commune	*Bacteroidetes*	2.80	Coastal	2.42	0.00
Nonlabens sp. MB-3u-79	*Bacteroidetes*	2.80	Coastal	2.42	0.00
Cellulophaga baltica	*Bacteroidetes*	2.77	Coastal	2.28	0.00
Flavobacterium sp. GENT11	*Bacteroidetes*	2.78	Coastal	2.36	0.00
Aegicerativicinus sediminis	*Bacteroidetes*	2.74	Coastal	2.15	0.00
Cellulophaga algicola	*Bacteroidetes*	2.74	Coastal	2.24	0.00
Flavobacterium sp. GENT5	*Bacteroidetes*	2.77	Coastal	2.38	0.00
Epilithonimonas vandammei	*Bacteroidetes*	2.70	Coastal	2.04	0.00
Muricauda aquimarina	*Bacteroidetes*	2.70	Coastal	2.09	0.00
Flavobacterium indicum	*Bacteroidetes*	2.71	Coastal	2.16	0.00
Flavobacterium johnsoniae	*Bacteroidetes*	2.73	Coastal	2.28	0.00
Maribacter sp. HTCC2170	*Bacteroidetes*	2.72	Coastal	2.22	0.00
Flavobacterium sp. SHINM13	*Bacteroidetes*	2.74	Coastal	2.32	0.00
Aquimarina sp. TRL1	*Bacteroidetes*	2.71	Coastal	2.17	0.00
Muricauda oceani	*Bacteroidetes*	2.75	Coastal	2.33	0.00
Flavobacterium anhuiense	*Bacteroidetes*	2.72	Coastal	2.26	0.00
Bacteroides ovatus	*Bacteroidetes*	2.76	Coastal	2.41	0.00
Bacteroides thetaiotaomicron	*Bacteroidetes*	2.75	Coastal	2.41	0.00
Bacteroides xylanisolvens	*Bacteroidetes*	2.74	Coastal	2.39	0.00
Myroides phaeus	*Bacteroidetes*	2.65	Coastal	2.02	0.00
Flavobacterium gilvum	*Bacteroidetes*	2.70	Coastal	2.28	0.00
Flavobacterium sp. F-70	*Bacteroidetes*	2.69	Coastal	2.28	0.00
Flavobacterium sp. F-323	*Bacteroidetes*	2.70	Coastal	2.31	0.00
Galbibacter sp. BG1	*Bacteroidetes*	2.67	Coastal	2.19	0.00
Flavobacterium nackdongense	*Bacteroidetes*	2.70	Coastal	2.32	0.00
Gramella forsetii	*Bacteroidetes*	2.65	Coastal	2.10	0.00
Bacteroides stercoris	*Bacteroidetes*	2.72	Coastal	2.41	0.00
Costertonia aggregata	*Bacteroidetes*	2.68	Coastal	2.28	0.00
Psychroflexus torquis	*Bacteroidetes*	2.63	Coastal	2.01	0.00
Maribacter sp. MJ134	*Bacteroidetes*	2.67	Coastal	2.26	0.00
Sediminicola sp. YIK13	*Bacteroidetes*	2.66	Coastal	2.22	0.00
Arenibacter algicola	*Bacteroidetes*	2.65	Coastal	2.18	0.00
Nonlabens dokdonensis	*Bacteroidetes*	2.64	Coastal	2.13	0.00
Flagellimonas maritima	*Bacteroidetes*	2.64	Coastal	2.19	0.00
Flavobacterium sp. MDT1-60	*Bacteroidetes*	2.65	Coastal	2.23	0.00
Nonlabens sp. Ci31	*Bacteroidetes*	2.65	Coastal	2.22	0.00
Flavobacterium fluviale	*Bacteroidetes*	2.63	Coastal	2.16	0.00
Flavobacterium branchiophilum	*Bacteroidetes*	2.62	Coastal	2.12	0.00
Flavobacterium sp. BB8	*Bacteroidetes*	2.64	Coastal	2.21	0.00
Aequorivita sublithincola	*Bacteroidetes*	2.62	Coastal	2.11	0.00

Gilvibacter sp. SZ-19	*Bacteroidetes*	2.66	Coastal	2.27	0.00
Flavobacterium sangjuense	*Bacteroidetes*	2.63	Coastal	2.17	0.00
Dokdonia sp. 4H-3-7-5	*Bacteroidetes*	2.62	Coastal	2.20	0.00
Capnocytophaga canimorsus	*Bacteroidetes*	2.60	Coastal	2.09	0.00
Flavobacterium sp. KBS0721	*Bacteroidetes*	2.61	Coastal	2.19	0.00
Dokdonia sp. PRO95	*Bacteroidetes*	2.61	Coastal	2.17	0.00
Flammeovirga kamogawensis	*Bacteroidetes*	2.59	Coastal	2.08	0.00
Flavobacterium nitrogenifigens	*Bacteroidetes*	2.59	Coastal	2.16	0.00
Nonlabens spongiae	*Bacteroidetes*	2.59	Coastal	2.12	0.00
Flavobacterium crocinum	*Bacteroidetes*	2.59	Coastal	2.13	0.00
Myroides odoratus	*Bacteroidetes*	2.57	Coastal	2.07	0.00
Flavobacterium sp. xlx-214	*Bacteroidetes*	2.55	Coastal	2.02	0.00
Flavobacterium sp. CLA17	*Bacteroidetes*	2.58	Coastal	2.18	0.00
Zobellia galactanivorans	*Bacteroidetes*	2.58	Coastal	2.19	0.00
Flavobacterium sp. LPB0248	*Bacteroidetes*	2.55	Coastal	2.11	0.00
Altibacter sp. ALE3EI	*Bacteroidetes*	2.55	Coastal	2.11	0.00
Flavobacterium sp. SLB02	*Bacteroidetes*	2.55	Coastal	2.14	0.00
Dokdonia sp. Dokd-P16	*Bacteroidetes*	2.55	Coastal	2.13	0.00
Muricauda ruestringensis	*Bacteroidetes*	2.53	Coastal	2.07	0.00
Flavobacterium kingsejongi	*Bacteroidetes*	2.52	Coastal	2.10	0.00
Dokdonia donghaensis	*Bacteroidetes*	2.51	Coastal	2.04	0.00
Gramella flava	*Bacteroidetes*	2.51	Coastal	2.07	0.00
Salegentibacter salegens	*Bacteroidetes*	2.50	Coastal	2.00	0.00
Pukyongia salina	*Bacteroidetes*	2.51	Coastal	2.08	0.00
Nonlabens marinus	*Bacteroidetes*	2.48	Coastal	2.03	0.00
Muriicola soli	*Bacteroidetes*	2.49	Coastal	2.07	0.00
Flavobacterium inviolabile	*Bacteroidetes*	2.48	Coastal	2.05	0.00
Cloacibacterium normanense	*Bacteroidetes*	2.46	Coastal	2.02	0.00
Draconibacterium orientale	*Bacteroidetes*	2.46	Coastal	2.10	0.00
Parabacteroides merdae	*Bacteroidetes*	2.46	Coastal	2.11	0.00
Bacteroides caccae	*Bacteroidetes*	2.46	Coastal	2.12	0.00
Bacteroides eggerthii	*Bacteroidetes*	2.43	Coastal	2.08	0.00
Bacteroides cellulosilyticus	*Bacteroidetes*	2.38	Coastal	2.01	0.00
Ilumatobacter coccineus	*Actinobacteria*	2.59	Coastal	2.22	0.00
Collinsella aerofaciens	*Actinobacteria*	2.39	Coastal	2.06	0.00

2. Human-impacted human pathogens retrieved from the comparable study between human-impacted coastal waters and pristine ocean systems (LEfSe, LDA score > 2, p < 0.05).

Human pathogen	Log10 average abundance	Enriched group	LDA	KW p-value
Abiotrophia defectiva	2.527911162	Coastal	2.100934942	7.69536E-08
Achromobacter xylosoxidans	3.514782689	Coastal	2.764909809	2.43235E-09
Acinetobacter baumannii	4.490165858	Coastal	3.381055428	0.000187673
Acinetobacter johnsonii	4.006884796	Coastal	3.363658786	2.80672E-18
Acinetobacter lwoffii	3.624247228	Coastal	2.936138907	9.34251E-17
Actinobacillus pleuropneumoniae	3.080256832	Coastal	2.057613212	0.047384925
Actinobacillus suis	3.047514095	Coastal	2.465246759	0.00075297
Aeromonas caviae	4.017710061	Coastal	3.632251956	1.07469E-27
Aeromonas hydrophila	3.635170465	Coastal	3.132545856	1.8925E-26
Aeromonas veronii	3.78950976	Coastal	3.3240059	5.14839E-28
Alcaligenes faecalis	3.464401654	Coastal	2.822956724	4.4451E-18
Bacteroides caccae	3.605405052	Coastal	3.234897898	4.82584E-23
Bacteroides eggerthii	3.571185217	Coastal	3.144112004	6.43603E-20
Bacteroides fragilis	3.955447088	Coastal	3.477327528	4.31535E-13
Bacteroides ovatus	3.901822745	Coastal	3.512718439	6.38074E-11
Bacteroides stercoris	3.882821902	Coastal	3.557828724	6.33072E-27
Bacteroides thetaiotaomicron	3.878828472	Coastal	3.503180226	3.4817E-21
Bacteroides uniformis	4.191893791	Coastal	3.870248919	3.59037E-27
Bifidobacterium dentium	2.828736102	Coastal	2.177967758	0.006767024
Bordetella avium	2.797477803	Coastal	2.263556353	4.58083E-17
Bordetella bronchiseptica	3.044539843	Coastal	2.000104004	1.9649E-05
Brevundimonas vesicularis	3.007884572	Coastal	2.430348824	7.54459E-06
Brucella anthropi	3.416338844	Coastal	2.739071028	9.36536E-12
Capnocytophaga gingivalis	3.332726576	Coastal	2.576614595	2.47964E-06
Capnocytophaga sputigena	3.488426416	Coastal	2.572554506	0.000810391
Cardiobacterium hominis	2.732730706	Coastal	2.053133394	2.56544E-09
Cedecea lapagei	2.54204442	Coastal	2.053235357	1.96878E-17
Cedecea neteri	3.086821982	Coastal	2.499437675	3.20388E-21
Citrobacter amalonaticus	2.848150183	Coastal	2.168900648	1.48652E-11
Citrobacter braakii	3.046313687	Coastal	2.477573429	3.80544E-16
Citrobacter farmeri	3.099359866	Coastal	2.585173172	5.17493E-15
Citrobacter freundii	4.108447114	Coastal	3.548794619	9.65016E-21
Citrobacter rodentium	2.576476528	Coastal	2.165885151	4.23042E-19
Citrobacter werkmanii	2.72985728	Coastal	2.085117598	6.04265E-10
Collinsella aerofaciens	3.520366533	Coastal	3.177662321	1.55849E-16

Comamonas testosteroni	3.406207736	Coastal	2.740830218	1.66646E-20
Corynebacterium argentoratense	2.23274545	Coastal	2.203340622	4.31535E-13
Corynebacterium jeikeium	2.961903073	Coastal	2.190889788	0.000120408
Corynebacterium striatum	3.041648757	Coastal	2.327910401	1.55281E-05
Corynebacterium urealyticum	2.727992586	Coastal	2.225170621	3.73446E-10
Delftia acidovorans	3.213027487	Coastal	2.377849544	5.91119E-07
Edwardsiella hoshinae	2.641793582	Coastal	2.156988705	7.07766E-16
Eggerthella lenta	2.621118404	Coastal	2.00478789	0.001128391
Eikenella corrodens	2.706433251	Coastal	2.144969226	3.41278E-15
Enterobacter asburiae	3.19530589	Coastal	2.606712077	2.92803E-19
Enterobacter cancerogenus	2.775863727	Coastal	2.19501791	2.40743E-15
Enterobacter cloacae	3.772985655	Coastal	3.214986451	3.71014E-19
Enterobacter hormaechei	4.010165345	Coastal	3.191594043	7.16658E-11
Escherichia coli	4.933431624	Coastal	4.049310399	5.04958E-08
Ewingella americana	2.781716234	Coastal	2.288615259	2.71701E-20
Gordonia terrae	2.863354932	Coastal	2.314640921	8.01573E-05
Haemophilus parainfluenzae	3.723784799	Coastal	2.301627907	0.042137396
Hafnia alvei	3.34873434	Coastal	2.667359232	8.35656E-16
Kingella denitrificans	2.681235643	Coastal	2.263363884	1.88882E-21
Klebsiella oxytoca	3.242101572	Coastal	2.658284544	1.50489E-23
Klebsiella pneumoniae	4.831257138	Coastal	4.267347731	1.27188E-12
Kluyvera ascorbata	2.495771434	Coastal	2.191889689	3.97365E-18
Moraxella bovis	2.891306996	Coastal	2.036814351	5.98176E-06
Moraxella osloensis	3.794363343	Coastal	3.167960349	1.42851E-21
Morganella morganii	3.723284993	Coastal	3.004015302	3.52107E-12
Mycobacterium marinum	2.751666846	Coastal	2.144967121	1.63411E-05
Neisseria flavescens	2.141245485	Coastal	2.128296021	2.14933E-06
Neisseria meningitidis	3.33502711	Coastal	2.726591584	1.20119E-20
Neisseria mucosa	2.735971908	Coastal	2.216032247	4.2039E-15
Neisseria sicca	2.66104047	Coastal	2.107415427	2.9776E-11
Pantoea agglomerans	3.297402914	Coastal	2.574660305	3.54122E-16
Plesiomonas shigelloides	3.282294112	Coastal	2.618900755	7.20092E-18
Prevotella denticola	2.683908558	Coastal	2.138777978	6.47134E-13
Prevotella ruminicola	2.782168237	Coastal	2.018728137	1.63656E-05
Proteus mirabilis	3.879136405	Coastal	2.865130296	7.18611E-05
Providencia stuartii	3.584490703	Coastal	2.969187506	2.93469E-08
Pseudomonas aeruginosa	4.396764556	Coastal	3.474753012	1.01528E-05
Pseudomonas alcaligenes	3.374817134	Coastal	2.876374024	1.17701E-13
Pseudomonas fluorescens	3.929322168	Coastal	3.304595028	1.16904E-18
Pseudomonas putida	4.053803604	Coastal	3.190798904	5.90436E-11
Pseudomonas stutzeri	4.196325128	Coastal	3.217779416	7.54459E-06
Rahnella aquatilis	3.089382484	Coastal	2.478908619	1.29657E-18
Ralstonia insidiosa	3.00381834	Coastal	2.359406838	8.12984E-12
Rhodococcus erythropolis	2.893378121	Coastal	2.205945853	1.25884E-06

Rhodococcus fascians	3.052283653	Coastal	2.473465477	4.57299E-07
Salmonella bongori	3.305088266	Coastal	2.637423323	0.016894973
Serratia marcescens	3.591969232	Coastal	2.944374715	3.35732E-18
Serratia odorifera	2.617226439	Coastal	2.176801033	6.07993E-15
Serratia plymuthica	2.974969285	Coastal	2.309187964	4.55463E-14
Serratia proteamaculans	2.681196053	Coastal	2.187919125	5.64222E-19
Serratia rubidaea	2.755092233	Coastal	2.154763598	6.07147E-13
Stenotrophomonas maltophilia	3.818326672	Coastal	3.079401769	1.86095E-13
Streptococcus salivarius	3.197165044	Coastal	2.049809375	0.000596171
Sutterella wadsworthensis	2.797617102	Coastal	2.310730956	2.71824E-16
Tatumella ptyseos	2.831022079	Coastal	2.262331862	6.46764E-17
Vibrio alginolyticus	4.237944274	Coastal	3.742500456	6.33526E-13
Vibrio cholerae	4.555462808	Coastal	4.081622681	2.40953E-16
Vibrio cincinnatiensis	3.416480423	Coastal	2.772573768	1.07843E-11
Vibrio fluvialis	3.933074758	Coastal	3.571791527	2.62186E-23
Vibrio furnissii	3.445837338	Coastal	3.000654472	1.9107E-25
Vibrio mimicus	3.7396393	Coastal	3.297455721	1.12655E-13
Vibrio parahaemolyticus	4.295052683	Coastal	3.893297461	2.34401E-22
Vibrio vulnificus	3.923017781	Coastal	3.359013267	1.37822E-23
Yersinia frederiksenii	3.261366448	Coastal	2.241804018	0.000373555
Yersinia kristensenii	3.298779299	Coastal	2.664383984	5.05611E-17
Yersinia pestis	4.415277094	Coastal	3.996417824	6.95238E-19
Yersinia pseudotuberculosis	2.882691683	Coastal	2.209989668	2.43235E-09
Yersinia ruckeri	3.007545115	Coastal	2.204957847	6.46807E-07

3. Human-impacted ARG subtypes retrieved from the comparable study between human-impacted coastal waters and pristine ocean systems (LEfSe, LDA score > 2, p < 0.05).

ARG subtype	Log10 average abundance	Enriched group	LDA	KW p-value
bacitracin__bacA	4.65	Coastal	4.04	2.78797E-27
kasugamycin__kasugamycin resistance protein ksgA	4.54	Coastal	4.10	4.50555E-22
unclassified__cAMP-regulatory protein	4.41	Coastal	4.02	7.2824E-22
multidrug__mexT	4.30	Coastal	3.80	5.71909E-17
multidrug__mexF	4.18	Coastal	3.68	5.71324E-16
multidrug__TolC	4.14	Coastal	3.63	8.11734E-23
beta-lactam__PBP-1A	4.17	Coastal	3.55	2.21407E-22
trimethoprim__dfrA1	4.09	Coastal	3.32	1.0601E-07
multidrug__multidrug_transporter	3.96	Coastal	3.15	0.00577456
multidrug__mdtK	3.93	Coastal	3.26	5.22194E-05
multidrug__mexW	3.98	Coastal	3.24	8.29603E-12
tetracycline__tet35	3.91	Coastal	3.36	4.95058E-19
carbomycin__carA	3.91	Coastal	3.45	3.73146E-25
vancomycin__vanS	3.82	Coastal	3.37	2.78084E-22
vancomycin__vanH	3.86	Coastal	3.45	5.95617E-26
quinolone__qnrS	3.55	Coastal	3.26	1.63083E-20
beta-lactam__penA	3.72	Coastal	3.28	2.34401E-22
macrolide-lincosamide-streptogramin__srmB	3.81	Coastal	3.42	6.04388E-28
multidrug__acrB	3.68	Coastal	3.06	3.6985E-08
chloramphenicol__catB	3.76	Coastal	3.17	5.85101E-16
macrolide-lincosamide-streptogramin__oleB	3.70	Coastal	3.25	1.84064E-24
macrolide-lincosamide-streptogramin__tlcC	3.69	Coastal	3.32	1.30121E-27
macrolide-lincosamide-streptogramin__vatB	3.57	Coastal	3.01	3.86091E-20
fosmidomycin__rosA	3.44	Coastal	2.94	0.027776856
chloramphenicol__cat_chloramphenicol acetyltransferase	3.56	Coastal	3.15	4.34209E-25
macrolide-lincosamide-streptogramin__msrC	3.50	Coastal	2.96	9.27111E-18
beta-lactam__PBP-1B	3.46	Coastal	3.04	2.92803E-19
tetracycline__tetA	3.52	Coastal	2.63	0.003249653
polymyxin__mcr-4	3.43	Coastal	3.05	8.81478E-18
tetracycline__tetO	3.48	Coastal	2.87	8.89641E-17
tetracycline__tetB	3.48	Coastal	3.12	5.48959E-28
multidrug__mexE	3.33	Coastal	2.80	7.85297E-07
tetracycline__tet41	3.44	Coastal	2.67	8.31184E-05

tetracycline__tetQ	3.27	Coastal	2.37	5.83575E-05
rifamycin__rifampin monooxygenase	3.44	Coastal	2.62	0.000225456
multidrug__emrE	3.35	Coastal	2.38	0.008691205
multidrug__ykkD	3.25	Coastal	2.77	1.00873E-05
vancomycin__vanC	3.36	Coastal	2.58	1.24057E-06
polymyxin__mcr-5	3.36	Coastal	2.93	5.05425E-20
multidrug__mexB	3.25	Coastal	2.64	7.69536E-08
macrolide-lincosamide-streptogramin__vgaE	3.34	Coastal	2.79	1.55351E-21
polymyxin__mcr-3	3.24	Coastal	2.86	1.13414E-17
macrolide-lincosamide-streptogramin__msrA	3.33	Coastal	2.91	2.29427E-25
macrolide-lincosamide-streptogramin__macA	3.12	Coastal	2.73	3.53986E-05
beta-lactam__class B beta-lactamase	3.12	Coastal	2.82	1.94029E-08
multidrug__acrA	3.09	Coastal	2.58	0.001415158
unclassified__DNA-binding_protein_H-NS	3.12	Coastal	2.83	1.11569E-14
tetracycline__tetW	3.18	Coastal	2.72	2.87925E-24
macrolide-lincosamide-streptogramin__vgaD	3.16	Coastal	2.47	3.3686E-05
macrolide-lincosamide-streptogramin__ermF	3.03	Coastal	2.73	3.63367E-17
macrolide-lincosamide-streptogramin__vatE	3.11	Coastal	2.61	4.94152E-15
tetracycline__tetracycline_resistance_protein	3.13	Coastal	2.31	1.4146E-06
multidrug__mexI	3.11	Coastal	2.43	1.33412E-08
multidrug__mexD	3.06	Coastal	2.59	2.22802E-18
polymyxin__icr-Mo	3.03	Coastal	2.62	4.34378E-16
macrolide-lincosamide-streptogramin__ermB	2.93	Coastal	2.64	4.23635E-12
multidrug__mdtF	2.97	Coastal	2.38	1.93089E-07
vancomycin__vanD	3.06	Coastal	2.17	7.971E-07
sulfonamide__sul1	2.97	Coastal	2.47	0.002526819
multidrug__adeJ	2.95	Coastal	2.35	6.05512E-05
multidrug__mdtC	2.99	Coastal	2.44	5.32144E-14
beta-lactam__CfxA2	2.91	Coastal	2.61	2.47622E-14
aminoglycoside__aadA	2.90	Coastal	2.60	1.14069E-13
macrolide-lincosamide-streptogramin__lsa	2.94	Coastal	2.60	3.76175E-24
multidrug__adeC	3.00	Coastal	2.60	1.17424E-21
beta-lactam__OXA-209	3.00	Coastal	2.67	4.44947E-18
sulfonamide__sul2	3.00	Coastal	2.35	6.15485E-10
aminoglycoside__aadE	2.89	Coastal	2.51	2.07637E-09
fosfomycin__fosX	3.04	Coastal	2.58	4.31533E-10
tetracycline__tet39	2.93	Coastal	2.42	2.21366E-13
beta-lactam__class A beta-lactamase	2.83	Coastal	2.36	1.78432E-05
multidrug__mdtB	2.85	Coastal	2.28	9.94955E-12
beta-lactam__VEB-1	2.71	Coastal	2.42	1.74668E-11
macrolide-lincosamide-streptogramin__mefA	2.73	Coastal	2.44	1.08782E-15
macrolide-lincosamide-streptogramin__vatG	2.87	Coastal	2.40	5.64138E-19
multidrug__emrD	2.84	Coastal	2.49	1.22844E-06
multidrug__smeD	2.79	Coastal	2.32	1.64055E-05

macrolide-lincosamide-streptogramin__vatC	2.84	Coastal	2.20	2.3248E-05
multidrug__mdtL	2.79	Coastal	2.51	2.24409E-14
multidrug__major_facilitator_ superfamily_transporter	2.74	Coastal	2.30	0.002593547
fosfomycin__fosA	2.81	Coastal	2.46	6.74691E-08
fosmidomycin__rosB	2.73	Coastal	2.31	2.63908E-06
multidrug__bicyclomycin-multidrug_efflux_protein_bcr	2.72	Coastal	2.19	5.08299E-07
multidrug__mdtE	2.66	Coastal	2.21	0.019419564
multidrug__adeB	2.71	Coastal	2.12	1.99987E-08
tetracycline__otrA	2.82	Coastal	2.30	1.03192E-13
tetracycline__tetX6	2.68	Coastal	2.36	2.39407E-17
tetracycline__tet37	2.62	Coastal	2.32	2.32872E-11
trimethoprim__dfrA17	2.49	Coastal	2.21	4.38028E-14
polymyxin__mcr-1.5	2.71	Coastal	2.34	4.09248E-20
aminoglycoside__aac(6')-I	2.56	Coastal	2.20	3.62924E-09
aminoglycoside__aph(6)-I	2.55	Coastal	2.23	3.76451E-13
tetracycline__tetS	2.68	Coastal	2.15	4.16638E-14
unclassified__bacterial regulatory protein LuxR	2.62	Coastal	2.31	3.24174E-18
vancomycin__vanY	2.57	Coastal	2.24	3.54676E-15
multidrug__mdfA	2.49	Coastal	2.16	5.14102E-06
macrolide-lincosamide-streptogramin__lnuB	2.57	Coastal	2.26	5.14882E-26
trimethoprim__dfrA14	2.41	Coastal	2.11	4.49857E-08
multidrug__oprN	2.68	Coastal	2.30	1.16544E-18
aminoglycoside__aph(3")-I	2.45	Coastal	2.15	2.405E-09
aminoglycoside__bifunctional aminoglycoside N-acetyltransferase and aminoglycoside phosphotransferase	2.41	Coastal	2.12	3.80099E-11
trimethoprim__dfrB6	2.46	Coastal	2.16	3.11256E-08
macrolide-lincosamide-streptogramin__vgaB	2.53	Coastal	2.09	3.61823E-11
beta-lactam__LCR-1	2.33	Coastal	2.00	0.001113039
macrolide-lincosamide-streptogramin__lnuA	2.38	Coastal	2.09	3.72993E-08
unclassified__sdiA	2.44	Coastal	2.12	3.08328E-13
vancomycin__vanU	2.35	Coastal	2.08	5.09217E-08
macrolide-lincosamide-streptogramin__ermC	2.41	Coastal	2.12	0.00081327
polymyxin__mcr-2.2	2.48	Coastal	2.06	1.41234E-15
polymyxin__icr-Mc	2.49	Coastal	2.14	5.84111E-20
tetracycline__tetX2	2.44	Coastal	2.13	2.4995E-21
chloramphenicol__chloramphenicol exporter	2.41	Coastal	2.05	4.9631E-11
polymyxin__mcr-2.1	2.51	Coastal	2.11	1.22734E-15
multidrug__mdtG	2.32	Coastal	2.01	1.65779E-05
tetracycline__tet40	2.34	Coastal	2.06	2.51667E-15
tetracycline__tetX	2.45	Coastal	2.04	2.8569E-18
polymyxin__eptA	2.46	Coastal	2.09	4.43397E-23

4. Human-impacted ARG hosts retrieved from the comparable study between human-impacted coastal waters and pristine ocean systems (LEfSe, LDA score > 2, $p < 0.05$).

ARG host	Pathogenicity	Log10 average abundance	Enriched group	LDA	KW p-value
Acinetobacter baumannii	Pathogenic	3.27	Coastal	2.96	0.01
Acinetobacter johnsonii	Pathogenic	3.03	Coastal	2.74	0.04
Aeromonas caviae	Pathogenic	3.49	Coastal	3.09	0.02
Bacteroides fragilis	Pathogenic	3.66	Coastal	3.36	0.00
Escherichia coli	Pathogenic	3.54	Coastal	3.24	0.02
Prevotella intermedia	Pathogenic	3.10	Coastal	2.76	0.01
Staphylococcus aureus	Pathogenic	3.00	Coastal	2.69	0.04
Streptococcus pneumoniae	Pathogenic	2.90	Coastal	2.55	0.04
Vibrio alginolyticus	Pathogenic	3.79	Coastal	3.45	0.00
Vibrio furnissii	Pathogenic	3.05	Coastal	2.73	0.04
Vibrio mimicus	Pathogenic	3.32	Coastal	3.05	0.04
Vibrio vulnificus	Pathogenic	3.54	Coastal	3.24	0.04
[Eubacterium] rectale	Non-pathogenic	3.05	Coastal	2.77	0.04
[Haemophilus] ducreyi	Non-pathogenic	3.06	Coastal	2.79	0.03
Aeromonas salmonicida	Non-pathogenic	3.20	Coastal	2.91	0.01
Aliarcobacter butzleri	Non-pathogenic	3.11	Coastal	2.72	0.04
Aliivibrio fischeri	Non-pathogenic	3.05	Coastal	2.69	0.03
Aliivibrio wodanis	Non-pathogenic	3.12	Coastal	2.80	0.01
Arcobacter sp. L	Non-pathogenic	3.29	Coastal	2.98	0.03
Colwellia psychrerythraea	Non-pathogenic	3.63	Coastal	3.29	0.02
Colwellia sp. PAMC 21821	Non-pathogenic	3.57	Coastal	3.28	0.03
Cronobacter muytjensii	Non-pathogenic	3.44	Coastal	3.21	0.04
Flavobacterium commune	Non-pathogenic	3.16	Coastal	2.85	0.04
Geoalkalibacter subterraneus	Non-pathogenic	4.02	Coastal	3.71	0.00
Glaciecola nitratireducens	Non-pathogenic	3.38	Coastal	3.12	0.04
Glaciecola sp. 4H-3-7+YE-5	Non-pathogenic	3.55	Coastal	3.16	0.01
Gynuella sunshinyii	Non-pathogenic	3.25	Coastal	2.93	0.02
Halomonas sp. HL-93	Non-pathogenic	3.13	Coastal	2.79	0.04
Marinobacter psychrophilus	Non-pathogenic	3.17	Coastal	2.89	0.04
Marivivens sp. JLT3646	Non-pathogenic	3.35	Coastal	2.95	0.04
Methylophilales bacterium MBRSF5	Non-pathogenic	3.80	Coastal	3.47	0.02
Oleiphilus messinensis	Non-pathogenic	3.55	Coastal	3.20	0.05
Oleispira antarctica	Non-pathogenic	3.98	Coastal	3.65	0.00
Parabacteroides sp. CT06	Non-pathogenic	2.99	Coastal	2.71	0.01
Paraburkholderia xenovorans	Non-pathogenic	3.47	Coastal	3.16	0.00
Phaeobacter gallaeciensis	Non-pathogenic	3.72	Coastal	3.36	0.00
Phaeobacter piscinae	Non-pathogenic	3.36	Coastal	3.09	0.04

Phaeobacter porticola	Non-pathogenic	3.08	Coastal	2.76	0.02
Phocaeicola dorei	Non-pathogenic	3.01	Coastal	2.71	0.02
Planktomarina temperata	Non-pathogenic	3.88	Coastal	3.52	0.00
Planococcus kocurii	Non-pathogenic	3.51	Coastal	3.17	0.04
Pseudoalteromonas arctica	Non-pathogenic	4.13	Coastal	3.81	0.00
Pseudoalteromonas atlantica	Non-pathogenic	3.95	Coastal	3.53	0.03
Pseudoalteromonas carrageenovora	Non-pathogenic	3.23	Coastal	2.93	0.01
Pseudoalteromonas issachenkonii	Non-pathogenic	3.61	Coastal	3.24	0.05
Pseudoalteromonas piratica	Non-pathogenic	3.66	Coastal	3.31	0.00
Pseudoalteromonas sp. 13-15	Non-pathogenic	3.91	Coastal	3.43	0.00
Pseudoalteromonas sp. SM9913	Non-pathogenic	3.86	Coastal	3.57	0.04
Pseudoalteromonas tetraodonis	Non-pathogenic	3.89	Coastal	3.55	0.01
Pseudomonas cichorii	Non-pathogenic	3.35	Coastal	3.05	0.00
Pseudomonas oleovorans	Non-pathogenic	3.60	Coastal	3.25	0.03
Pseudosulfitobacter pseudonitzschiae	Non-pathogenic	3.67	Coastal	3.38	0.00
Psychromonas sp. CNPT3	Non-pathogenic	3.23	Coastal	2.95	0.03
Rhodobacteraceae bacterium	Non-pathogenic	3.59	Coastal	3.32	0.03
Rhodovulum sp. P5	Non-pathogenic	3.43	Coastal	3.20	0.04
Shewanella frigidimarina	Non-pathogenic	3.78	Coastal	3.44	0.04
Shewanella marisflavi	Non-pathogenic	3.39	Coastal	3.06	0.01
Sulfitobacter alexandrii	Non-pathogenic	3.52	Coastal	3.23	0.00
Thalassolituus oleivorans	Non-pathogenic	4.18	Coastal	3.78	0.00
Thiomonas intermedia	Non-pathogenic	3.04	Coastal	2.78	0.04
Thiomonas sp. CB2	Non-pathogenic	3.24	Coastal	2.93	0.04
Tritonibacter mobilis	Non-pathogenic	3.29	Coastal	2.96	0.01
uncultured bacterium BD_contig00402	Non-pathogenic	2.98	Coastal	2.71	0.02
Vibrio coralliilyticus	Non-pathogenic	3.31	Coastal	2.97	0.02
Vibrio mediterranei	Non-pathogenic	3.33	Coastal	2.97	0.04
Vibrio tapetis	Non-pathogenic	2.98	Coastal	2.68	0.02

5. Human-impacted mobile ARGs retrieved from the comparable study between human-impacted coastal waters and pristine ocean systems (LEfSe, LDA score > 2, $p < 0.05$).

Mobile ARG subtype	Pathogenicity	Log10 average abundance	Enriched group	LDA	KW p-value
aminoglycoside__aadA	Pathogenic	3.65	Coastal	3.50	0.05
aminoglycoside__aph(3")-I	Pathogenic	3.30	Coastal	3.48	0.05
aminoglycoside__aph(6)-I	Pathogenic	3.50	Coastal	3.58	0.02
aminoglycoside__bifunctional aminoglycoside N-acetyltransferase and aminoglycoside phosphotransferase	Pathogenic	3.15	Coastal	3.47	0.05
beta-lactam__CARB-8	Pathogenic	4.11	Coastal	3.83	0.03
beta-lactam__CfxA2	Pathogenic	4.15	Coastal	3.93	0.00
kasugamycin__kasugamycin resistance protein ksgA	Pathogenic	4.97	Coastal	4.30	0.01
MLS__ermB	Pathogenic	3.61	Coastal	3.51	0.03
MLS__ermF	Pathogenic	3.69	Coastal	3.49	0.03
multidrug__qacEdelta1	Pathogenic	5.13	Coastal	4.70	0.00
quinolone__qnrA	Pathogenic	3.61	Coastal	3.47	0.03
quinolone__qnrS	Pathogenic	4.26	Coastal	3.93	0.00
sulfonamide__sul1	Pathogenic	3.77	Coastal	3.64	0.01
tetracycline__otrA	Pathogenic	3.86	Coastal	3.64	0.03
tetracycline__tet32	Pathogenic	3.15	Coastal	3.47	0.05
tetracycline__tetQ	Pathogenic	4.00	Coastal	3.80	0.00
unclassified__cAMP-regulatory protein	Pathogenic	4.94	Coastal	4.20	0.02
unclassified__DNA-binding_ protein_H-NS	Pathogenic	3.65	Coastal	3.43	0.05
vancomycin__vanS	Pathogenic	4.18	Coastal	3.81	0.01

MLS, macrolide-lincosamide-streptogramin.

6. Human-impacted pathogenic ARGs retrieved from the comparable study between human-impacted coastal waters and pristine ocean systems (LEfSe, LDA score > 2, $p < 0.05$).

Pathogenic ARG subtype	Log10 average abundance	Enriched group	LDA	KW p-value
aminoglycoside__aadA	3.60	Coastal	3.47	0.05
aminoglycoside__bifunctional aminoglycoside N-acetyltransferase and aminoglycoside phosphotransferase	3.57	Coastal	3.50	0.02
bacitracin__bacA	4.61	Coastal	4.22	0.02
beta-lactam__CfxA2	4.28	Coastal	4.03	0.00
beta-lactam__VEB-3	3.51	Coastal	3.39	0.03
kasugamycin__kasugamycin resistance protein ksgA	4.70	Coastal	4.34	0.02
macrolide-lincosamide-streptogramin__ermB	4.07	Coastal	3.81	0.01
macrolide-lincosamide-streptogramin__ermF	3.99	Coastal	3.72	0.03
multidrug__mdtL	3.33	Coastal	3.42	0.05
multidrug__mexW	4.24	Coastal	3.86	0.02
quinolone__qnrA	3.81	Coastal	3.68	0.01
quinolone__qnrS	4.76	Coastal	4.41	0.00
tetracycline__otrA	4.05	Coastal	3.82	0.01
tetracycline__tet39	3.19	Coastal	3.43	0.05
tetracycline__tetM	3.70	Coastal	3.52	0.01
tetracycline__tetQ	4.41	Coastal	4.12	0.00
trimethoprim__dfrA17	3.77	Coastal	3.62	0.02
unclassified__cAMP-regulatory protein	5.21	Coastal	4.63	0.02
unclassified__DNA-binding_protein_H-NS	3.73	Coastal	3.55	0.05
vancomycin__vanS	3.69	Coastal	3.60	0.02

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Ongoing Manuscripts from the Research Project

1. **Zhang, X.H.,** Jin, L., Xie, J.W., Sun, X.H., Yan, Z.H., Leung, M.Y., Zhang, T., Li, X.D. Mariculture reshapes human pathogens and antibiotic resistomes in farmed fish. Environmental Research. 2025 (To be submitted).
2. **Zhang, X.H.**, Jin, L., Li, C.C., Xie, J.W., Leung, M.Y., Zhang, T., Li, X.D. Anthropogenic reconstruction of coastal bacteriomes and antibiotic resistomes and the linkage to community-based infections. (In preparation).
3. Yan, Z.H., **Zhang, X.H.,** Fat, S.T., Xie, J.W., Chiou, J.C., Yu, J., Li, X.D. Food-residue-level antibiotics promote mucosal colonization of foodborne antibiotic-resistant Staphylococcus aureus in a simulated human gut. Gut Microbes. 2025 (Submitted).
4. Yan, Z.H., Xie, J.W., Jin L., He, T.T., **Zhang, X.H.,** Li, X.D. Steam cooking methods promote the transfer of viable antibiotic-resistant pathogens from water into air. Environment & Health. 2025 (revision requested).