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Unveiling Mobilizable Multiresistance Clusters in Marine Bacteria †

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Abstract: The occurrence and spread of antibiotic resistance have become a pressing global health concern. Understanding the genetic elements that facilitate the dissemination of antibiotic resistance genes (ARGs) in marine environments is crucial for effective microbial surveillance and management strategies. This study aimed to reveal the presence of mobilizable multiresistance clusters, consisting of ARGs associated with mobile genetic elements (MGEs), in marine bacterial communities. Water samples were collected from two beaches in Jeju, South Korea, and screened to identify multi-drug resistant bacteria. A total of 20 bacterial isolates were selected for whole genome sequencing, and through comprehensive genomic analysis, we identified and characterized nine such clusters primarily composed of betalactams, aminoglycosides, and tetracycline ARGs associated with MGEs like IS6, IS9, and Tn3. Additionally, an extensive analysis of 900 marine bacterial genomes from the National Center for Biotechnology Information (NCBI) database was conducted to gain a broader perspective. Our results provide valuable insights into the prevalence and diversity of mobilizable multiresistance clusters in marine bacterial communities.

Keywords: antimicrobial resistance; mobile genetic elements; multiresistance clusters; genetic dissemination

1. Introduction

Antibiotic resistance is a global concern in the realm of human health. The emergence of community-acquired infections caused by resistant bacteria has amplified interest in natural environments (ref). These natural environments encompass a diverse range of ecosystems, including animals, soils, glaciers, and marine habitats, all of which serve as pivotal reservoirs for antibiotic resistance genes (ARGs). Despite the typically dilute nature of marine waters, they have evolved into significant reservoirs for antibiotic resistance. This phenomenon is primarily driven by escalating anthropogenic activities, which facilitate the introduction of antibiotic-resistant bacteria and residual antibiotics into marine ecosystems. Consequently, it is imperative to acquire a comprehensive understanding of the dissemination patterns of antibiotic resistance among marine bacteria. This knowledge is fundamental for the implementation of effective antibiotic control measures and the development of strategies to address this pressing issue [1].

Mobile genetic elements (MGEs), such as insertion sequences and transposons, possess the remarkable capacity to capture ARGs from bacterial chromosomes and subsequently transfer them horizontally, either through plasmids or phages, to other bacterial species. In this study, our objective is to illuminate the prevalence of ARGs within marine bacteria and their genomic colocation with MGEs. This research promises to yield valuable insights into the potential for ARG dissemination among marine bacteria, which, in turn, may exacerbate the spread of antibiotic resistance within marine ecosystems.

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2. Materials and Methods

Water samples were collected in triplicate from Jungmun beach, located in Jeju, South Korea. Culturable bacteria were isolated from these samples using Marine agar (BD Difco) by incubating at 30°C. Subsequently, isolates were sub-cultured on trypticase soy agar (BD Difco) with 3% additional salt content at 30°C. A total of 100 distinct colonies were selected for plasmid carriage determination based on their resistance to 14 different antibiotics, as determined through a disk diffusion test. Isolates exhibiting resistance to three or more distinct antibiotics were then screened for plasmids using alkaline lysis method. This process identified 60 plasmid-carrying isolates whose plasmid sizes were estimated using gel electrophoresis. Twenty isolates were selected from this group based on the distinct sizes of their plasmids for further analysis.

DNA from these 20 isolates was extracted using QIAamp DNA Mini kit (Qiagen, Hilden, Germany), and DNA quantity was assessed using Qubit™ (Thermo Fisher Scientific, USA). Short-read sequencing was conducted using Illumina Hiseq 2500 (Macrogen, Seoul, South Korea), while long-read sequencing employed MinION (Oxford nanopore technology). Raw reads from and MinION were subjected to quality trimming using fastp [2] and Filtlong (https://github.com/rrwick/Filtlong/), respectively. Assembly was performed using Unicycler [3], and taxonomic identification was carried out via the RDP classifier [4]. Further, functional analysis for antibiotic resistance genes was executed using AMRFinder [5], and mobile genetic elements (MGEs), including plasmids, prophages, transposons, and integrases were identified through plasmidFinder [6], phaster [7], and Isescan [8].

To complement our findings, we retrieved 900 marine bacterial genomes from National Center for Biotechnology Information (NCBI) for additional analysis (Figure 1). The assembly and analysis of these genomes followed the same procedures as mentioned above."

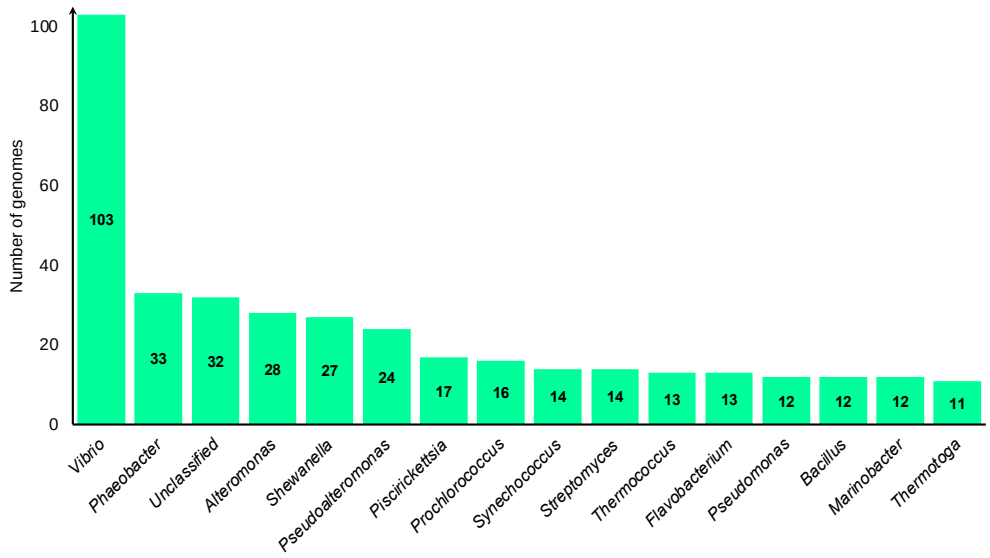

Figure 1. Graph representing prominent bacterial genomes at the genus level, featuring more than 10 species, retrieved from NCBI.

3. Results and Discussion

Taxonomic analysis revealed that the isolated bacteria belonged to various species, including *Vibrio cholera* (6), *Vibrio alginolyticus* (4), *Vibrio parahaemolyticus* (3), *Phaeobacter inhibens* (3), *Aeromonas salmonicida* (2), and *Edwardsiella anguillarum* (2). Antibiotic susceptibility testing (AST) showed increased resistance to beta-lactam, aminoglycoside, and

tetracycline antibiotics, particularly within the *Vibrio* species, aligning with genotypically identified ARGs as shown in Table 1.

These antibiotics are extensively used in livestock farms for treatment and prophylactic purposes [9]. This suggests that the antibiotic residues from neighboring livestock farms may intensify selective pressure on marine bacteria, leading to heightened resistance to these antibiotics. Moreover, multi drug resistance (MDR) *Vibrio* species are significant pathogens for aquatic life and humans. Therefore, the resistance observed in these isolates may pose an increased risk to both human and animal health [10].

Table 1. The alignment of phenotypic resistance with the corresponding genotypic resistance in MDR marine bacteria.

Bacterial Taxonomy	Antibiotic Class	AST	ARG	Plasmids	Prophages
Vibrio cholerae	Beta-lactam	Ampicillin Imipenem Meropenem Ceftazidime	*blaTEM-1* *blaS1* *YEM-1*	IncFII_1 (2) IncH12A-1 (2) Unnamed plasmids (3)	PHAGE_Vibrio_K139 PHAGE_Vibrio_VP882 PHAGE_Klebsi_ST16 PHAGE_Escher_520873 (2)
	Aminoglycoside	Kanamycin	*cpxA*		
	Tetracycline	Oxytetracycline	*tetA*		
Vibrio alginolyticus	Beta-lactam	Ampicillin Imipenem Aztreonam	*blaOXA50* *MexR*	p3442-IMI-2c Unnamed plasmids (3)	PHAGE_Vibrio_K139 PHAGE_Vibrio_VfO3K6 (2) PHAGE_Vibrio_VCY (2) PHAGE_Erwini_vB_EhrS (2)
	Aminoglycoside	Kanamycin	*AAC(6′)-34*		
	Tetracycline	Oxytetracycline	*tetA, tetB*		
	Fluoroquinolones	Ciprofloxacin	*oqxA, oqxB*		
	Phenicol	Chloramphenicol	*Cat*		
Vibrio parahaemolyticus	Beta-lactam	Ampicillin Imipenem Aztreonam	*blaXCARB2* *MexR*	Unnamed plasmids (3)	PHAGE_Vibrio_K139 PHAGE_Klebsi_ST16 PHAGE_Klebsi_ST17 PHAGE_Staphy_Spbeta
	Aminoglycoside	Kanamycin	*AAC(6′)-34*		
	Tetracycline	Oxytetracycline	*tetB*		
	Polymyxin B	*Colistin*	*PmrC*		
Phaeobacter inhibens	Beta-lactam	Ampicillin	*blaTEM-1*	IncFII_1 Unnamed plasmids (4)	PHAGE_Entero_mEp237 PHAGE_Entero_mEp235
	Fluoroquinolones	Levofloxacin	*gyrA, gyrB*		
	Phenociol	Florfenicol	*floR*		
Aeromonas salmonicida	Aminoglycoside	Kanamycin	*cpxA*	IncFII_1 Unnamed plasmids (2)	PHAGE_Entero_mEp237 (2) PHAGE_Klebsi_ST17 PHAGE_Salmon_118970_sal3 (2)
	Tetracycline	Oxytetracycline	*tetB*		

Edwardsiella anguillarum	Beta-lactam	Ampicillin	*blaTEM-1*	Unnamed plasmids (2)	PHAGE_Entero_mEp235
	Polymyxin B	Colistin	*eptA*		

The concordance observed between phenotypic and genotypic resistance implies the potential utility of whole genome sequencing for resistance surveillance across diverse environments [11]. Genomic analysis unveiled the presence of 25 intact prophages in the MDR isolates, with PHAGE_Vibrio_K139 (n=3) and PHAGE_Entero_mEp237 (3) being the most prevalent. The majority of these prophages were classified within the Siphoviridae family. (Figure 2). These findings inspired us to further investigate the functional roles of these prophages and their potential influence on the prevalence of antibiotic resistance within marine bacterial species.

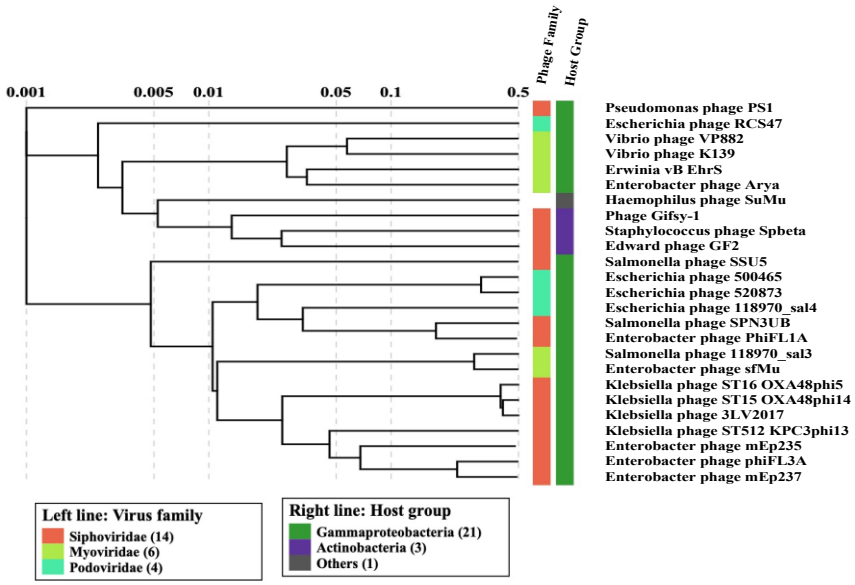

Figure 2. The taxonomic classification and host range of prophages encoded by marine bacterial genomes (900).

Furthermore, our analysis unveiled the presence of 24 plasmid sequences encoded within these bacterial genomes. These plasmids exhibited diversity, with the majority categorized as Unnamed plasmids (17), while others belonged to the IncFII_1 (4), IncH12A-1 (2), and p3442-IMI-2c (1) categories (see Table 1). The co-occurrence of plasmids and prophages within these genomes is a noteworthy observation, as it implies a potential interplay between mobile genetic elements and their role in shaping the genetic landscape of these marine bacterial species [12].

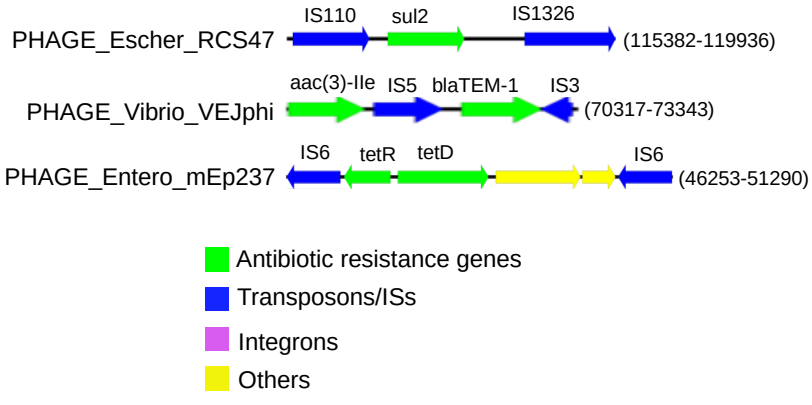

Figure 3. Three distinct associations between ARGs and MGEs were identified, each encoded within a unique prophage-like sequence. Notably, all three of these sequences were found to be prevalent among *Vibrio alginolyticus* isolates.

We identified a total of nine distinct associations between ARGs and MGEs within both prophage-like sequences (Figure 3) and plasmid contigs (Figure 4). Notably, MGEs such as IS6, IS5, and IS110 exhibited predominant associations with ARGs. These specific MGEs are recognized for their capacity to facilitate the transposition of associated genes within genomes, subsequently transferring them to other horizontally gene transfer (HGT) vehicles, including plasmids and prophages [13].

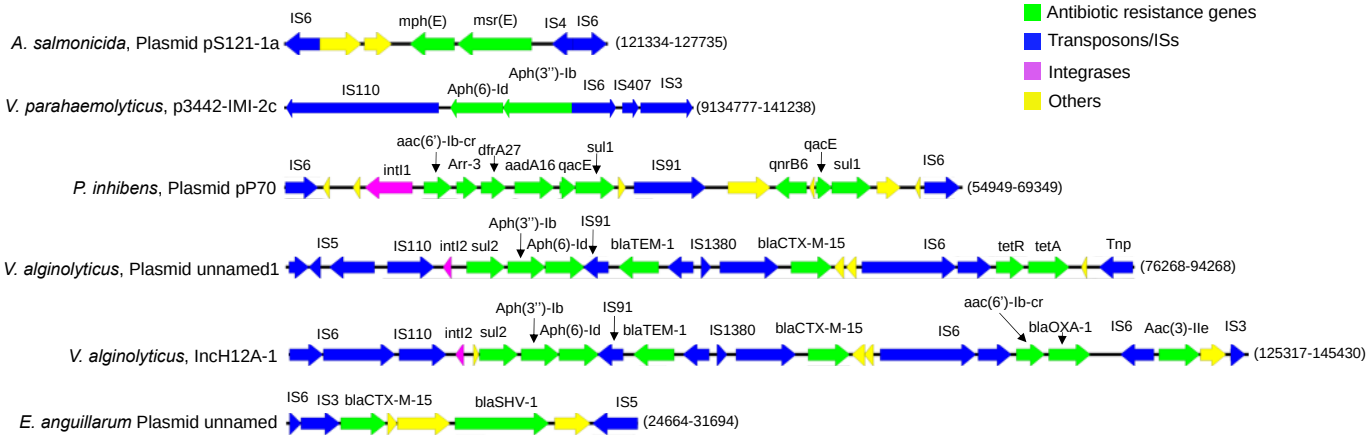

Figure 4. Mobile resistance clusters encoded by plasmid and prophage-like sequences: six distinctive ARG and MGE associations found within five distinct bacterial species.

Significantly, we have identified two prophage-like sequences, Vibrio phage VP882 and Vibrio phage VFO3k6, each encoding betalactam (*blaTEM-1*) and aminoglycoside (*aph(3'')-Ib*) genes, respectively, within various marine bacterial species (Figure 5). Notably, these genes exhibited 100% identical sequences across these diverse species. This highlights transduction as a key driver in the widespread distribution of ARGs by these prophages, showcasing their remarkable conservation. [14].

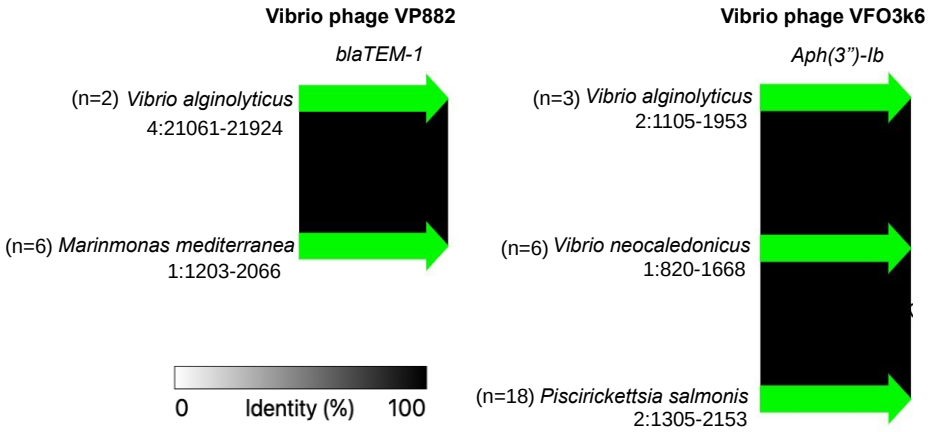

Figure 5. The sequence conservation of two aminoglycoside resistance genes encoded within prophages.

4. Conclusions

Our study sheds light on the intricate dynamics of antibiotic resistance in marine bacterial communities, revealing significant role of plasmids and prophages in ARGs spread. Future research should delve deeper into the functional roles of these MGEs.

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References

1. Marti, E.; Variatza, E.; Balcazar, J.L. The role of aquatic ecosystems as reservoirs of antibiotic resistance. *Trends Microbiol.* **2014**, *22*, 36–41.
2. Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. Fastp: An ultra-fast all-in-one fastq preprocessor. *Bioinformatics* **2018**, *34*, i884–i90. 10.1093/bioinformatics/bty560.
3. Wick, R.R.; Judd, L.M.; Gorrie, C.L.; Holt, K.E. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. *PLOS Computational Biology* **2017**; *13*, e1005595. 10.1371/journal.pcbi.1005595.
4. Lan, Y.; Wang, Q.; Cole, J.R.; Rosen, G.L. Using the rdp classifier to predict taxonomic novelty and reduce the search space for finding novel organisms. *PLoS One* **2012**, *7*, e32491. 10.1371/journal.pone.0032491.
5. Feldgarden, M.; Brover, V.; Haft, D.H.; Prasad, A.B.; Slotta, D.J.; Tolstoy, I.; et al. Validating the amrfinder tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. *Antimicrobial Agents and Chemotherapy* **2019**, *63*, 10.1128/aac.00483-19.
6. Carattoli, A.; Zankari, E.; García-Fernández, A.; Voldby Larsen, M.; Lund, O.; Villa, L.; et al. In silico detection and typing of plasmids using plasmidfinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* **2014**, *58*, 3895–903. 10.1128/aac.02412-14.
7. Arndt, D.; Grant, J.R.; Marcu, A.; Sajed, T.; Pon, A.; Liang, Y.; et al. Phaster: A better, faster version of the phast phage search tool. *Nucleic Acids Res.* **2016**, *44*, W16–21. 10.1093/nar/gkw387.
8. Xie, Z.; Tang, H. Isescan: Automated identification of insertion sequence elements in prokaryotic genomes. *Bioinformatics* **2017**, *33*, 3340–3347. 10.1093/bioinformatics/btx433.
9. Ghimpețeanu, O.M.; Pogurschi, E.N.; Popa, D.C.; Dragomir, N.; Drăgotoiu, T.; Mihai, O.D.; et al. Antibiotic use in livestock and residues in food-a public health threat: A review. *Foods* **2022**, *11*. 10.3390/foods11101430.
10. Huang, Z.; Anokyewaa, M.A.; Wang, J.; Jian, J.; Lu, Y. Pathogenicity and antibiotic resistance analysis of vibrio species found in coastal water at mainly beach of shenzhen, china. *Frontiers in Marine Science* **2022**, *9*, 980593.
11. Calero-Cáceres, W.; Ortuño-Gutiérrez, N.; Sunyoto, T.; Gomes-Dias, C.A.; Bastidas-Caldes, C.; Ramírez, M.S.; et al. Whole-genome sequencing for surveillance of antimicrobial resistance in ecuador: Present and future implications. *Rev Panam Salud Publica* **2023**, *47*, e8. 10.26633/rpsp.2023.8.
12. Khedkar, S.; Smyshlyaev, G.; Letunic, I.; Maistrenko, O.M.; Coelho, L.P.; Orakov, A.; et al. Landscape of mobile genetic elements and their antibiotic resistance cargo in prokaryotic genomes. *Nucleic Acids Res.* **2022**, *50*, 3155–3168. 10.1093/nar/gkac163.
13. Haudiquet, M.; de Sousa, J.M.; Touchon, M.; Rocha, E.P.C. Selfish, promiscuous and sometimes useful: How mobile genetic elements drive horizontal gene transfer in microbial populations. *Philos Trans R Soc Lond B Biol Sci.* **2022**, *377*, 20210234. 10.1098/rstb.2021.0234.
14. Schneider, C.L. Bacteriophage-mediated horizontal gene transfer: Transduction. In: Harper DR, Abedon ST, Burrowes BH, McConville ML, editors. Bacteriophages: Biology, technology, therapy. Cham: Springer International Publishing; 2021. p. 151–192.