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Abundance and diversity of antibiotic resistance genes and bacterial communities in the western Pacific and Southern Oceans

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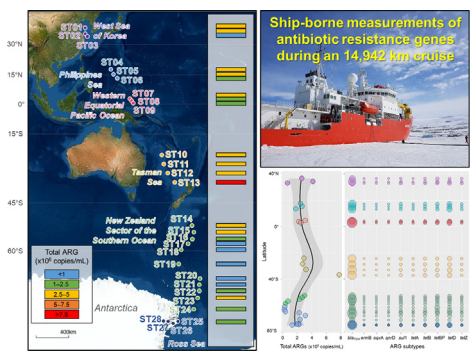
HIGHLIGHTS

- ARGs are widely distributed in the western Pacific and Southern Oceans.
- ARGs in the Southern Ocean are likely influenced by terrestrial sources.
- bla_{TEM} and *tet* were found to be dominant species for all samples.
- ARG abundance and related bacteria vary depending on the latitude.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated the abundance and diversity of antibiotic resistance genes (ARGs) and the composition of bacterial communities along a transect covering the western Pacific Ocean (36°N) to the Southern Ocean (74°S) using the Korean icebreaker R/V Araon (total cruise distance: 14,942 km). The relative abundances of ARGs and bacteria were assessed with quantitative PCR and next generation sequencing, respectively. The absolute abundance of ARGs was $3.0 \times 10^6 \pm 1.6 \times 10^6$ copies/mL in the western Pacific Ocean, with the highest value (7.8×10^6 copies/mL) recorded at a station in the Tasman Sea (37°S). The absolute abundance of ARGs in the Southern Ocean was 1.8-fold lower than that in the western Pacific Ocean, and slightly increased (0.7–fold) toward Terra Nova Bay in Antarctica, possibly resulting from natural terrestrial sources or human activity. β-Lactam and tetracycline resistance genes were dominant in all samples (88–99%), indicating that they are likely the key ARGs in the ocean. Correlation and network analysis showed that *Bdellovibrionota*, *Bacteroidetes*, *Cyanobacteria*, *Margulisbacteria*, and *Proteobacteria* were positively correlated with ARGs, suggesting that these bacteria are the most likely ARG carriers. This study highlights the latitudinal profile of ARG distribution in the open ocean system and provides insights that will help in monitoring emerging pollutants on a global scale.

1. Introduction

Antibiotic resistance in bacteria has become prevalent in response to the overuse of antibiotics (Baquero et al., 1998). Antibiotic resistance genes (ARGs) pose a serious and global threat to both public health and environmentally sustainable development (ICGoA, 2019). In

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2020, the US Centers for Disease Control and Prevention (CDC) reported that nearly three million people were infected with antibiotic resistant bacteria, with three-and-a-half million people dying yearly in the US from ARG-related infections (CDC, 2020). Once released into the environment, ARGs can be transferred among bacterial species, including fecal indicators (e.g., enterococci) and pathogens (Agersø et al., 2006; Iversen et al., 2004). Moreover, they cannot be completely removed from wastewater treatment plants (WWTPs) (Hernández et al., 2019), as maximum removal efficiencies of only 50.8–88.5% have been reported (Li et al., 2015; Rodriguez-Mozaz et al., 2015). Pathogenic bacteria harboring ARGs from WWTPs are typically discharged into marine environment by sewer overflows (Zhang et al., 2020). Thus, marine environments might serve as a channel for spreading pathogenic bacteria harboring ARGs to humans (Gao et al., 2018; Leonard et al., 2018). Owing to the severe threat to human health posed by bacteria harboring ARGs, it is essential to analyze and understand their distribution and diversity in marine environments.

Studies on marine ARGs have been conducted in various matrices, including marine sediments (Andersen et al., 1994; Goyal et al., 1979; Rahman et al., 2008; Stewart and Koditschek, 1980), estuaries and coastal waters (Carney et al., 2019; Chen et al., 2013; Fresia et al., 2019; Heuer et al., 2002; Jang et al., 2021; Leonard et al., 2018; Mudryk, 2005; Na et al., 2014; Nonaka et al., 2014; Zhang et al., 2020), and the oceans (Moore et al., 2020; Toth et al., 2010). Research on ARGs in marine waters situated close to WWTPs has determined the existence of various types of gentamicin resistance genes in treated WWTP effluent (Heuer et al., 2002), and significant correlations have been observed between ARGs and wastewater sources (e.g., WWTPs and combined sewer overflows) or adjacent coastal waters (Jang et al., 2021; Zhang et al., 2020). These suggest that ARGs can be used as an indicator for marine pollution. The concentrations of ARGs transferred to the ocean may be decreased by dilution, but can spread to the global marine environment (Gao et al., 2018). Nevertheless, as most studies are local (e.g., coastal areas strongly influenced by human activity), little is known about the distribution or diversity of ARGs in open ocean system on a global scale.

The abundance and diversity of ARGs in the ocean can be affected by several factors, including gene transfer between bacteria, the distance from land, and latitude. First, ARGs can be horizontally transferred by multiple mechanisms including conjugation, transformation, and transduction (Bengtsson-Palme et al., 2014; Furushita et al., 2003; Muniesa et al., 2013; Nonaka et al., 2014; Sullivan et al., 2013). Previous studies have reported that ARGs might be horizontally transferred by facilitation of the class 1 integron-integrase gene (*intI*1) on urban beaches by conjugating bacterial plasmids (Carney et al., 2019; Jang et al., 2021; Sullivan et al., 2013). The other factor is the distance from land. Indeed, the relative abundance of ARGs in coastal marine environments was reported to be 1–3 orders larger in magnitude than in the open ocean (Li et al., 2015; Suzuki et al., 2019; Yang et al., 2019). Moreover, ARG diversity has been reported differently depending on distance from land. For instance, the most abundant ARG in coastal waters adjacent to municipal sewage systems were sulfonamide- (Zhang et al., 2020) and β-lactam- (Fresia et al., 2019) resistance genes, whereas the multi-antibiotic resistance gene was most abundant in the North Pacific Gyre (Yang et al., 2019). Meanwhile, microbial communities harboring ARGs are diverse; both abundance and diversity vary, owing to differences in seawater temperatures dependent on latitude (Ibarbalz et al., 2019; Salazar et al., 2019; Sunagawa et al., 2015). If ARG has any correlation with bacterial abundance or diversity, ARG abundance and diversity also should vary by latitude. However, to the best of our knowledge, no research has been conducted on ARGs and the factors affecting them at different latitudes.

In this study, seawater samples were collected along a transect from the western Pacific Ocean (36°N) to the Southern Ocean (74°S) during the Korean icebreaker *R/V* Araon cruise. The aims of this study were 1) to explore the abundance and diversity of ARGs in seawater from the western Pacific Ocean (10,751 km of transect) to the Southern Ocean (4191 km of transect), 2) to investigate the impact of environmental parameters (e.g., water temperature, salinity, and distance from land), and 3) to profile bacterial communities correlated with ARGs.

2. Materials and methods

2.1. Sampling

In total, 28 seawater samples from a depth within 7 m were obtained during the *R/V* Aaron icebreaker (Korea Polar Research Institute, Korea) voyage from 31 October to 4 December 2019 (35 days) in the western Pacific Ocean and the Southern Ocean. The total distance of the cruise was 14,942 km, and the sampling sites are shown in Fig. 1. Exclusive economic zones (EEZs) were excluded from sampling, and distances between each sampling stations and adjacent land are shown in Table 1.

Sampling in the western Pacific Ocean was conducted at 13 sites (designated ST01 to ST13, Fig. 1) over a distance of 10,751 km, covering the West Sea of Korea (WS), the Philippine Sea (PS), Western Equatorial Pacific Ocean (WEPO), and Tasman Sea (TS) in the western Pacific Ocean. Sampling at ST01–03 was conducted in the WS immediately after departure from Incheon Port (Korea) on 31 October 2019. ST03 was closer to South Korea (Jeju Island) and only 208 km from Japan; therefore, we hypothesized that this station would provide samples contaminated by pollutants from both Korea and Japan. Samples were collected at three sites near the PS (ST04–06), situated 1625 ± 57 km away from the Philippines islands. Three other sites (ST07–09) near the WEPO were selected that were possibly influenced by Papua New Guinea. The last four samples of Cruise 1 were collected between the TS (ST10–13). The TS is located an average of 1126 km from Australia and New Zealand. All sites in the western Pacific Ocean were situated 51–1662 km (average 850 km) away from the nearest land (Table 1).

In the Southern Ocean, 15 samples (ST14–ST28, Fig. 1) were collected during a 4191 km long cruise, including the New Zealand sector of the Southern Ocean (NZSSO) and Ross Sea (RS). Sampling on the *R/V* Araon, which departed from Littleton Port, New Zealand, on 25 November 2019, was performed outside New Zealand's EEZ (ST14–18; 1836 ± 676 km from New Zealand). Jangbogo Station is located in the Terra Nova Bay, west of southeast Antarctica, with stations of Germany (Gondwana Camp) and Italy (Mario Zucchelli Station) operating within a 10 km radius of Jangbogo Station. Sea ice was present after ST19, and ST25 was taken from a polynya. In general, sampling sites in the RS were between 0.42 km (ST28; Jangbogo Station) and 299 km (ST25) from Antarctica (Table 1).

Bulk seawater samples were collected from each location in flexible plastic containers using an in-vessel pump. Containers were washed sequentially with 70% ethanol, ultrapure water, and seawater immediately before sample collection. After sampling, between 150 mL and 4 L of seawater was filtered through a membrane filter (0.45 μm, nylon, Whatman) using vacuum filtration prior to quantifying the ARGs. For bacterial diversity analysis, total bacterial cells were trapped on 3.0 μm and 0.2 μm membrane filters, for particle-attached bacteria and free-living bacteria, respectively, using vaccum. Filters were sterilized by autoclaving before use and kept in an in-vessel clean bench. After filtration, each filter was stored in a 2 mL e-tube (Axygen, USA) at −80 °C for DNA extraction, and blank filters were stored in the same way without filtration. All samples were transferred to a laboratory on dry ice in a refrigerated vehicle after unloading in Korea. At each sampling stations, each two filters were collected for ARGs, particle-attached and free-living bacteria, and blanks. After The environmental parameters including water temperature and salinity measured at each station with a probe to determine the conductivity–temperature–depth profile of the vessel (Table 1). The distance from each station to the nearest land was measured using Google Earth Pro (ver. 7.3.3.7786).

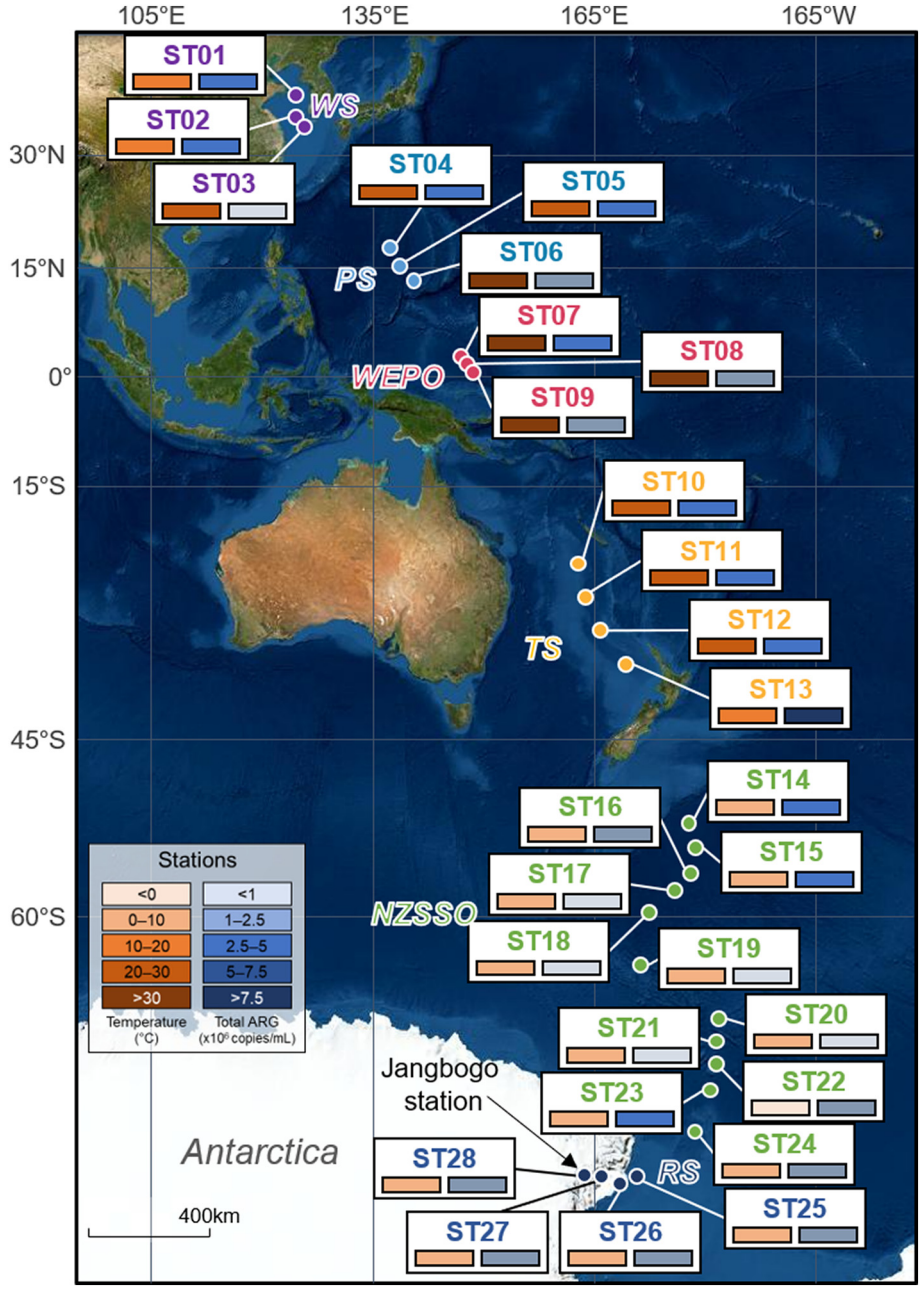

Fig. 1. Geographic distribution of surface seawater temperature and total ARG abundance at 28 sampling stations. ST01–03 (West Sea of Korea; WS, purple), ST04–06 (Philippine Sea; PS, blue), ST07–09 (Western Equatorial Pacific Ocean; WEPO, red), ST10–13 (Tasman Sea; TS, yellow), ST14–24 (New Zealand sector of the Southern Ocean; NZSSO; green), and ST25–28 (Ross Sea; RS, navy). There are two boxes in each station, the left box indicates the temperature and the right box indicates the total ARG abundance. The temperature was fractionated into 5 groups: <0 °C (blue), 0–10 °C (green), 10–20 °C (yellow), 20–30 °C (orange), and >30 °C (red). And the absolute abundance of total ARG also categorized into 5 groups: $<1 \times 10^6$ copies/mL (blue), 1×10^6–2.5×10^6 copies/mL (green), 2.5×10^6–5×10^6 copies/mL (yellow), 5×10^6–7.5×10^6 copies/mL (orange), and $>7.5 \times 10^6$ copies/mL (red).

2.2. DNA extraction and quantitative polymerase chain reaction (qPCR) for quantifying ARGs

Total DNA from each sample was extracted with membrane filters using the GeneAll Exgene™ Soil SV Kit (GeneAll Biotechnology, South Korea) in accordance with the manufacturer's protocol. Eluted DNA (50 μL) was stored at −20 °C, and the ARGs, 16S rRNA, and *intI*1 were analyzed by qPCR amplification. The *intI*1 gene has been used as a proxy for horizontal gene transfer in previous studies (Dong et al., 2019; Wang et al., 2014a; Zhu et al., 2013). ARGs included five sub-types of tetracycline resistance genes (*tet*A, *tet*B, *tet*BP, *tet*D, and *tet*Z), one sulfonamide-resistance gene (*sul*1), one macrolide resistance gene (*erm*B), one *β*-lactam resistance gene (bla_{TEM}), one quinolone-resistance gene (*qnr*D), and one multi-antibiotic resistance gene (*oqx*A). These ARGs were selected based on those previously indentified in seawater in previous studies (Na et al., 2014; Lu et al., 2019; Yang et al., 2019; Li et al., 2020). We quantified 16S rRNA to normalize ARGs for quantification, due to the difference between the abundance of background bacteria and extraction efficiency. Accordingly, the relative abundance of ARG to total bacteria was quantified using the 16S rRNA gene. The optimal annealing temperature was designed according to the qPCR system and primer requirements (Table S1). Standard curves for each ARGs were generated using a 10-fold serial dilution of known concentrations of plasmid DNA. Each qPCR reaction was performed in triplicate.

Table 1
Water quality parameters and distance from adjacent countries at 7-m depth of each sampling station.

Region		Station	Sampling date (UTC)	Latitude	Longitude	Water temperature (°C)	Salinity (PSU)	Adjacent country	Distance from the country (km)
Western Pacific Ocean	West Sea of Korea (WS)	ST01	2019.10.31. (13:16)	36°13 N	125°50E	18.67	31.88	South Korea China	60.72 304.14
		ST02	2019.11.01. (00:02)	33°54 N	125°48E	19.60	32.76	South Korea Japan China	77.84 281.66 433.56
		ST03	2019.11.01. (06:04)	32°46 N	126°23E	24.75	33.98	South Korea (Jeju Island) Japan China	51.39 207.64 431.82
	Philippine Sea (PS)	ST04	2019.11.05. (00:00)	17°14 N	138°03E	29.59	34.23	Philippines	1654.79
		ST05	2019.11.05. (12:01)	15°06 N	139°35E	29.98	34.40	Philippines	1559.26
		ST06	2019.11.05. (23:30)	13°06 N	141°01E	30.59	33.99	Philippines	1661.66
	Western Equatorial Pacific Ocean (WEPO)	ST07	2019.11.08. (03:00)	04°16 N	147°29E	30.27	33.97	Papua New Guinea	960.68
		ST08	2019.11.08. (07:59)	03°25 N	148°08E	30.06	33.92	Papua New Guinea	916.97
		ST09	2019.11.08. (13:00)	02°32 N	148°49E	30.54	34.07	Papua New Guinea	868.54
Southern Ocean	Tasman Sea (TS)	ST10	2019.11.14. (20:21)	24°25S	163°01E	24.16	35.64	Australia New Zealand	1007.85 1453.44
		ST11	2019.11.15. (19:09)	28°46S	163°51E	22.60	35.70	Australia New Zealand	998.62 1044.41
		ST12	2019.11.16. (19:00)	33°04S	165°49E	20.80	35.68	New Zealand	650.92
		ST13	2019.11.17. (18:49)	37°05S	168°53E	18.32	35.42	New Zealand	428.24
	New Zealand sector of the Southern Ocean (NZSSO)	ST14	2019.11.26. (19:51)	53°04S	178°18E	9.33	33.84	New Zealand	960.53
		ST15	2019.11.27. (03:34)	54°31S	179°13E	9.62	33.87	New Zealand	1118.02
		ST16	2019.11.27. (11:00)	55°50S	179°27E	9.02	33.85	New Zealand	1240.62
		ST17	2019.11.27. (19:00)	57°09S	177°36E	7.80	33.77	New Zealand	1302.25
		ST18	2019.11.28. (07:03)	59°04S	174°59E	6.90	33.60	New Zealand	1437.13
		ST19	2019.11.28. (19:03)	61°30S	174°10E	4.59	33.54	New Zealand	1681.35
		ST20	2019.11.29. (07:04)	63°47S	173°17 W	1.74	33.32	New Zealand	2190.23
		ST21	2019.11.29. (14:09)	65°09S	172°44 W	0.43	33.41	New Zealand	2326.20
		ST22	2019.11.29. (18:29)	66°00S	172°19 W	−0.18	33.50	New Zealand	2412.24
		ST23	2019.11.30. (07:17)	68°11S	171°28 W	2.27	33.65	New Zealand	2632.76
		ST24	2019.12.01. (03:04)	71°05S	171°54 W	1.11	34.09	New Zealand	2898.66
	Ross Sea (RS)	ST25	2019.12.02. (16:19)	74°45S	177°41E	0.70	34.18	Antarctica	299.52
		ST26	2019.12.03. (00:33)	75°15S	171°48E	1.25	34.20	Antarctica	196.94
		ST27	2019.12.03. (07:29)	74°55S	166°41E	0.86	33.95	Antarctica	47.97
		ST28	2019.12.04. (21:19)	74°37S	164°14E	0.45	34.55	Antarctica	0.42

2.3. Bacterial community analysis

2.3.1. DNA extraction and PCR amplification

Filters collected at each station were shredded before extracting template DNA using a FastDNA™ Spin Kit for Feces (MP Biomedicals, USA), according to the manufacturer's protocol. Tools used to shred the filters (scissors, tweezers, and glass dishes) were sterilized and shredding was performed under aseptic conditions. The extracted DNA templates were amplified by nested PCR for the 16S rRNA gene using 27F (5′-AGAGTTTGATCMTGG-3′) and 1492R (5′-TACCTTGTTACGACTT-3′) primers in a first round and 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 926R (5′-CCGYCAATTYMTTTRAGTTT-3′) primers with barcodes in a second round. The 50 μL DNA template mix (DNA template, 5 μL; 10 pM F/R primers, 5 μL; DNAase-free water, 40 μL) was amplified using 30 cycles with AccuPower® PCR Premix (Bioneer, Korea) in a T100 Thermal Cycler (Bio-Rad, USA) using the following conditions: 3 min at 94 °C, 1 min at 94 °C, 1 min at 56 °C, 2 min at 72 °C, and 5 min at 72 °C. A 5 μL sample of each PCR product underwent electrophoresis with a 100 bp DNA size marker (AccuLadder™, Bioneer, Korea). Each PCR run was conducted in triplicate.

2.3.2. Next-generation sequencing and analysis of sequence data

PCR products were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Lithuania). Finally, 16S rRNA gene amplification was quantified using a Qubit™ 4 Fluorometer (Invitrogen, USA) calibrated with a Qubit™ 1X dsDNA HS Assay Kit (Invitrogen, OR). The same amount of DNA was pooled for next-generation sequencing, performed with a paired-end cycle type on a MiSeq (Illumina) using the TruSeq Nano DNA Sample Preparation Kit (Illumina). Raw FASTQ files were trimmed and demultiplexed using Trimmomatic (ver. 0.39) and Mothur (Schloss et al., 2009). Clusters of operational taxonomic units (OTUs) were classified using the RDP Classifier and the 16S rRNA database based on a 97% similarity with Qiime2 (ver. 2020.8) (Bolyen et al., 2019).

2.4. Statistical analysis

Principal component analysis (PCA) was performed to assess correlations between latitude, water temperature, salinity, distance from land, and *intI*1 and the absolute abundance of total ARGs (i.e., the sum of 10 ARGs in this study). The values of total ARGs and *intI*1 were log-scaled owing to their high skewness, 1.4 and 2.8, respectively. Negative values were used for distance to clearly recognize relationships, and latitude was applied as an absolute value. Rarefied richness is an index used to statistically analyze species abundance; it is calculated as described by Sanders (1968). The Shannon diversity index ist a measure of the uniform distribution of species within a cluster, that is, the diversity. These two indices have been widely applied to investigate the abundance and diversity of microbial communities in various aquatic environments. In this study, the *factoextra* and *vegan* packages in *R* (ver. 4.0.2) were used to calculate PCA, rarefied richness, and the Shannon index.

Correlations among ARGs, environmental parameters, and bacteria at the phylum level were analyzed using Pearson's correlation coefficients. Correlations were visualized using the ggplot2 package in *R*. Network analyses were conducted to determine the relationships among ARGs, environmental parameters, and bacteria at the genus level using an extended local similarity analysis based on pattern similarity (Durno et al., 2013). Local similarity (LS) was calculated using the fastLSA software package based on significant correlation factors (p-value < 0.05). Pairwise co-occurrence patterns based on LS were visualized using Cytoscape version 3.8.2 software. In both networks, OTUs with maximum relative abundance of more than 0.1% were selected, and 254 of the 322 OTUs in the particle-attached bacteria and 166 of the 262 OTUs in the free-living bacteria were used for analysis.

3. Results & discussion

3.1. Abundance and diversity of ARGs along the transect

3.1.1. Abundance of ARGs

Total ARG abundances (i.e., sum of 10 ARGs) along a transect from the western Pacific Ocean (including WS, PS, WEPO, and TS) to the Southern Ocean (including NZSSO and RS) are shown in Fig. 2. Total ARG abundance in all samples ranged from 2.5×10^5 to 7.8×10^6 copies/mL, with an average of $2.3 \times 10^5 \pm 6.3 \times 10^5$ copies/mL. Total ARG abundance in the western Pacific Ocean averaged $3.0 \times 10^6 \pm 1.6 \times 10^6$ copies/mL, 1.8-fold higher than in the Southern Ocean ($1.7 \times 10^6 \pm 1.0 \times 10^6$ copies/mL), with the lowest abundance at ST20 (2.5×10^5 copies/mL) (Fig. 2a). There was a significant difference between TS and NZSSO (Fig. 2b), possibly a consequence of the 1.9–31 times higher total ARG abundance in ST13 (TS) than in other sampling stations. In the present study, the western Pacific Ocean has greater exposure to human activities from land (e.g., South Korea, Japan, Philippines, Papua New Guinea, Australia, and New Zealand) than does the Southern Ocean (Table 1). As shown by several previous studies, this result suggests that high ARG abundance may associate with human activity or anthropogenic sources of pollution (Imran et al., 2019; Moore et al., 2020; Zhang et al., 2020). Meanwhile, total ARG abundance showed an increasing trend from the farthest sampling site toward the land in the Southern Ocean. Total ARG abundance in NZSSO was significantly lower than that of TS ($p < 0.05$) with the highest value at ST23 (3.1×10^6 copies/mL) and the lowest at ST20 (2.2×10^6 copies/mL), which were 2633 km and 2190 km from New Zealand, respectively (Fig. 2). Although ARG abundance in RS did not differ significantly from those in other seas, there was a trend of increasing ARG abundance as we headed toward Antarctica. Overall, ARG abundance in the Southern Ocean was lower than that in the western Pacific Ocean but increased toward Antarctica, which may be caused by natural terrestrial sources or human activities in the research stations. This result is consistent with previous reports that ARGs are produced by human activities (e.g., residence for research purposes or tourism) and by incomplete wastewater treatment in the Antarctic environment (Hernández et al., 2016, 2019; Jara et al., 2020; Miller et al., 2009; Opazo-Capurro et al., 2019; Rabbia et al., 2016; Wang et al., 2016).

3.1.2. Diversity of ARGs

The abundance of 10 ARGs is shown in Fig. 3a. The abundance of bla_{TEM} was the highest ($1.7 \times 10^6 \pm 1.2 \times 10^6$ copies/mL) of the target ARGs, with a relative value ($4.2 \times 10^{-3} \pm 2.0 \times 10^{-3}$ copies/16S rRNA) similar to that of river (Guan et al., 2018) and gray (Porob et al., 2020) water. At all sampling stations, bla_{TEM} accounted for the highest ARG percentage (53.7–87.2%), followed by *tet*BP (3.48–25.5%), *tet*D (3.19–24.3%), *sul*1 (0.91–12.2%), *tet*B (1.01–6.28%), *tet*Z (0.34–4.19%), *tet*A (0.01–0.80%), *oqx*A (0.00–0.14%), *erm*B (0.00–0.05%), and *qnr*D (0.01–0.03%). Moreover, bla_{TEM} showed a strong positive correlation with total ARG abundance across the stations ($r^2 = 0.99$; Fig. S1).The β-lactam resistance gene, bla_{TEM} confers resistance to penicillin, and is one of the most prevalent ARGs in the environment along with enteric bacteria (Narciso-Da-Rocha et al., 2014). Accordingly, bla_{TEM} could be 1) naturally occurring in the ocean or from terrestrial sources without anthropogenic exposure (e.g., soil) or 2) associated with the construction of the research station (i.e., human activities). For instance, *bla* group genes have been found even in the pristine Indian Ocean, (Calero-Cáceres and Balcázar, 2019), Alaskan soil (Allen et al., 2009), and Arctic permafrost (D'Costa et al., 2011). In Antarctic soil, 177 naturally occurring ARGs have been reported for natural antibiotics (Van Goethem et al., 2018). Those were distance from anthropogenic sources or human activity, suggesting that *bla* group genes may naturally occur in the ocean itself. In addition, Wang et al. (2016) and reported strong correlation between ARG abundance (including *bla* group genes) and distance from Jangbogo Station. The study assessed ARGs (including *bla* group genes) for 4 years before and during construction of the Jangbogo research station. The result suggests that human activities for the construction of the research station had a strong influence on ARG distribution in the environment. Both abundance and diversity of bla_{TEM} tended to increase toward Antarctica in present study; however, it is difficult to determine whether they are influenced by human activities. Since there are previous studies of *bla* group genes in pristine environments and around Jangbogo Station, both natural and human origins should be considered as factors.

The diversity of ARGs indicated by the Shannon diversity index was not significantly different ($p > 0.05$) between the western Pacific Ocean and the Southern Ocean (Fig. 3b). Local differences were detected in the ARG content at several stations. The abundances of bla_{TEM} at ST03 (WS; 54.5%), ST06 (PS; 62.7%), and ST19–20 (NZSSO; 53.7%–59.7%) were significantly lower than those measured at the other stations (74.6% ± 6.9%, $p < 0.001$). Conversely, the abundances of *tet* group genes, sum of *tet*A, *tet*B, *tet*BP, *tet*D, and *tet*Z, were 1.7-fold more abundant at ST03, ST06, and ST19–20 than they were at other stations. Tetracycline is an inexpensive antibiotic that has been widely used for decades in a wide range of activities, including the treatment of human and veterinary diseases, aquaculture, and agriculture (Chopra and Roberts, 2001; Daghrir and Drogui, 2013); thus, *tet* group genes were the commonly detected ARG in human feces (Baron et al., 2018). Based on previous studies, terrestrial sources with human activity could be considered factors for the relatively higher *tet* group genes abundance measured at ST03, ST06, and ST19–20. Our

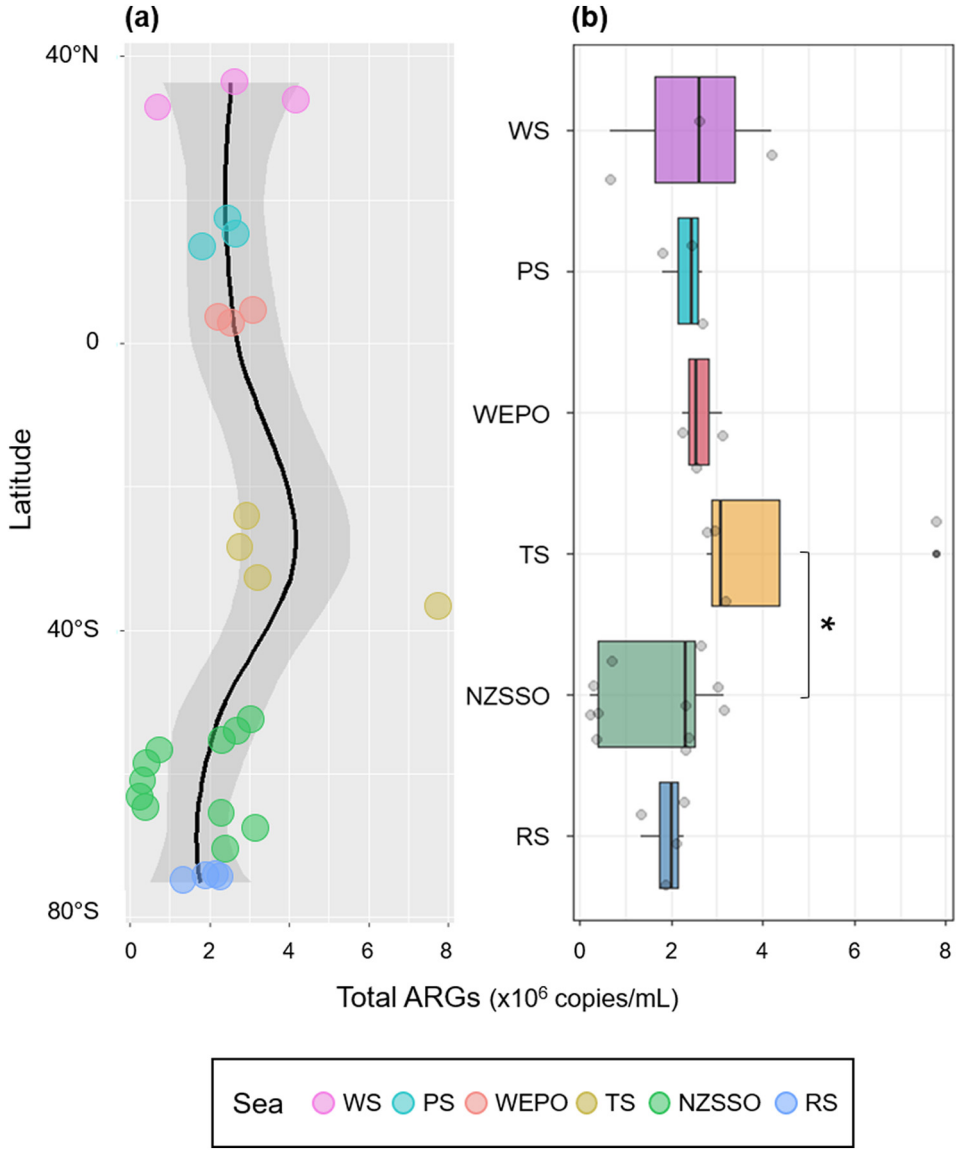

Fig. 2. Relative abundances ($\times 10^6$ copies/mL) of total ARGs according to (a) latitude and (b) sea in the West Sea of Korea (WS), Philippine Sea (PS), Western Equatorial Pacific Ocean (WEPO), Tasman Sea (TS), New Zealand sector of the Southern Ocean (NZSSO), and Ross Sea (RS), where * indicates significant difference (ANOVA, $p < 0.05$).

ARG diversity data indicate that differences in ARG diversity are likely to be determined by other environmental factors rather than latitude.

3.2. Impact of environmental parameters on the abundance and diversity of ARGs

PCA was carried out by dividing areas into the western Pacific and Southern Oceans to verify if environmental parameters and distance from land were related to the total ARG abundance. For the western Pacific Ocean, principal components 1 and 2 (PC1 & PC2) explained 71% of total variation (Fig. 4a and Table S4). Total ARGs contributed the most to PC2, whereas temperature and latitude contributed to PC1. Salinity contributed the most to PC2 along with total ARGs, indicating that salinity and total ARG abundance were positively correlated. Previous studies have reported negative correlations between salinity and ARGs or antibiotic resistance rates through studies conducted with bacteria in the laboratory (Wang et al., 2015) and in the field (Lu et al., 2015). These studies suggest that increased salinity reduces ARG abundance by hindering the spread of microorganisms carrying ARG in estuarine environments, where the difference in salinity is large (0.41–22.5 PSU) (Lu et al., 2015; Wang et al., 2015). Conversely, Zhang et al. (2019) reported a study in which environmental conditions such as high salts act as stressors, enhancing bacterial resistance to a wide range of antibiotics using modeling of coastal soils. In this study, salinities were high and narrow (34.03 ± 0.83 PSU), apparently increasing ARG abundance via stressors. Meanwhile, despite the large variation in distance between sampling stations and adjacent land in the western Pacific Ocean (876 ± 598 km), distance from land did not show significant correlation with total ARGs in this region. In the western Pacific Ocean, national differences in ARG distribution (i.e., Korea, Japan, Philippines, Papua New Guinea, Australia, and New Zealand) are likely to be different. Indeed, a report by WHO (2018) found that abundances and dominant types of antibiotics varied among these countries. Accordingly, different antibiotic usage probably affects both ARG occurrence on land and their release into the oceanic environment. Overall, in the western Pacific Ocean, high salinity condition seems to be an important environmental parameter influencing ARG abundance and diversity; therefore, other physical/chemical parameters should be more studied rather than distance from land.

In the Southern Ocean, PC2, with distance from land and salinity, contributed to total ARGs (Fig. 4b). Several previous studies have provided evidence that ARGs in the Southern Ocean are affected by both terrestrial sources and human activities (Jara et al., 2020; Miller et al., 2009; Opazo-Capurro et al., 2019), including, wastewater (Hernández et al., 2019;

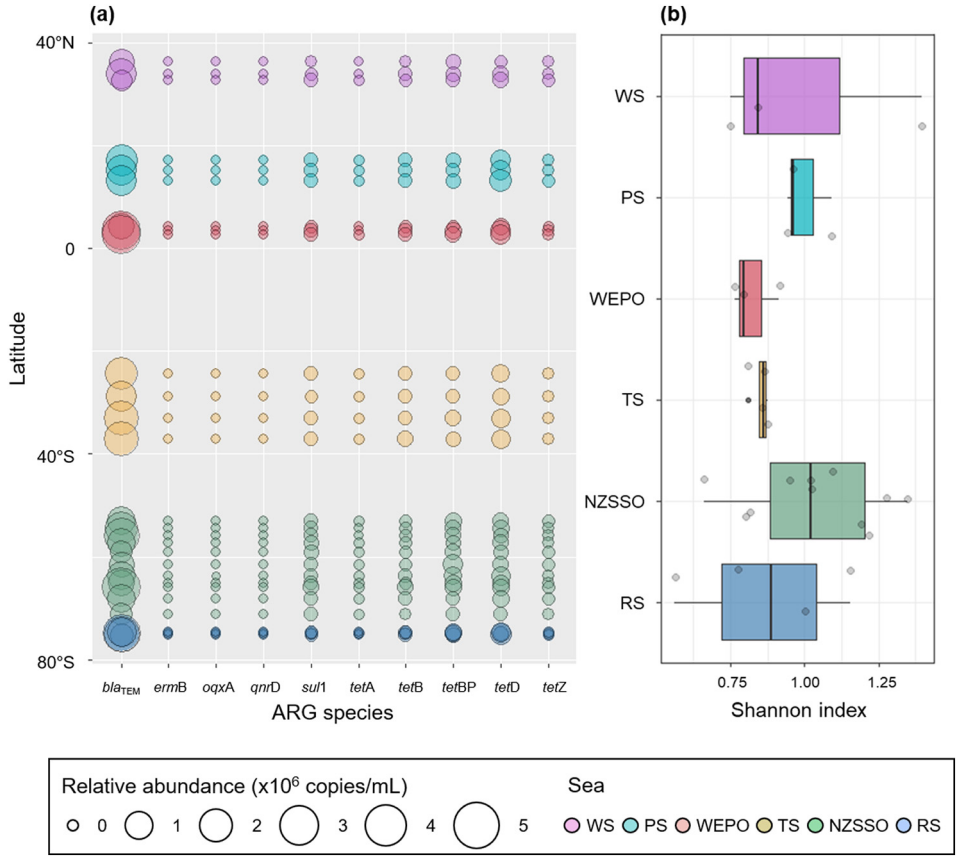

Fig. 3. (a) Relative abundance and (b) diversity of ARGs in the West Sea of Korea (WS), Philippine Sea (PS), Western Equatorial Pacific Ocean (WEPO), Tasman Sea (TS), New Zealand sector of the Southern Ocean (NZSSO), and Ross Sea (RS).

Rabbia et al., 2016), research, and tourism (Hernández et al., 2016; Wang et al., 2016). In particular, Wang et al. (2016) detected a total of 73 ARGs from soil samples at an 11 km distance between Gondwana Camp and Jangbogo Station in the Antarctic Terra Nova Bay, inferred pollutants from the construction of the research station as the likely source. Antarctic soil ARGs originating from terrestrial sources might affect nearby seawater in Antarctica, ultimately serving as an ARG reservoir. We hypothesized that six stations (ST14–19) in NZSSO and all stations in the RS (ST25–28) may be affected by terrestrial sources. Moreover, ARG abundance showed an increasing trend toward Palmer Station on Anvers Island, indicating that antibiotic resistance in Antarctica increases with human habitation (Miller et al., 2009). Therefore, this suggests that distance from land is a significant factor that determines the abundance of total ARGs in the Southern Ocean.

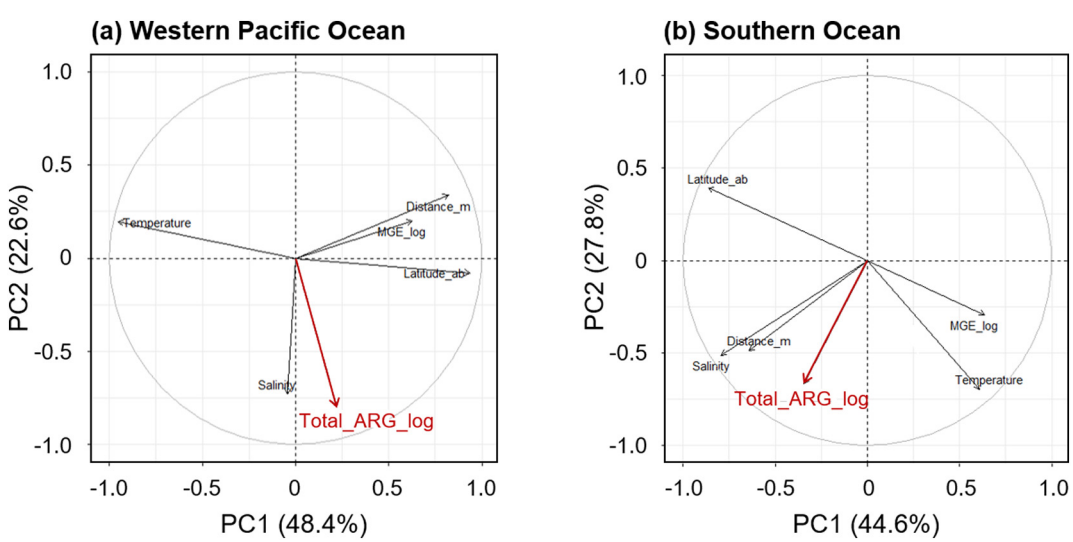

Fig. 4. Principal component analysis of total ARG, MGE, latitude, temperature, salinity, and distance from lands in (a) the Pacific Ocean and (b) the Southern Ocean. Total ARG and MGE values were log-scaled ('Total_ARG_log' and 'MGE_log'), distance took negative values ('Distance_m') for clear recognition, and absolute values were used for latitude ('Latitude_ab').

3.3. Impact of intI1 on ARG abundance and diversity

Horizontal gene transfer is well-known and its mechanism include conjugation, transformation, and transduction (Tazzyman and Bonhoeffer, 2014; Van Goethem et al., 2018; Dong et al., 2019). In this study, *intI*1 was quantified as a proxy for horizontal gene transfer (Dong et al., 2019; Wang et al., 2014a), since previous studies have reported that microbes and mobile gene elements including *intI*1 are the primary components affecting ARG profiles (Xiong et al., 2014; Zhao et al., 2019). The correlations between ARGs and *intI*1 were determined by PCA and Pearson's correlation (Fig. 4 and Table S5). PCA showed no correlation between total ARG abundance and *intI*1 in both the western Pacific and Southern Oceans (Fig. 4). However, with respect to correlations between individual ARGs and MGE, four ARGs (*tet*BP, *tetZ*, *sul*1, and *oqx*A) showed significant positive correlations ($p < 0.01$) with the *intI*1 gene (Table S5). This suggests that these four are more likely to be transferred horizontally among bacteria in marine environments (Zhang et al., 2017). In particular, *tet* group genes were observed to spread to pathogens (Sullivan et al., 2013; Wang et al., 2014b), and *sul* group genes were transferred from marine aquaculture farms through horizontal gene transfer (Muziasari et al., 2014). ARGs also have been found in the pristine Arctic soil, where they may have been spread horizontally (McCann et al., 2019). Indeed, it has been suggested that horizontal gene transfer was responsible for ARG transfer in,the Arctic, because the sampling stations were far from the contamination source (McCann et al., 2019). The abundance of these ARGs was low in total ARGs but was detected across all sampling stations in this study. Thus, in this study, ARG may have spread to pristine open seas through horizontal gene transfer, regardless of latitude.

3.4. Profiling bacterial communities related to ARGs

To investigate the potential ARG-related bacterial communities, heatmaps were generated based on Pearson's correlation coefficients at the phylum level (Fig. 5) and a network analysis at the genus level (Fig. 6). Bacterial communities are divided into two size groups according to Ortega-Retuerta et al. (2013) as follows: free-living (0.2–3.0 μm) and particle-attached (>3.0 μm). Several studies have shown that the latter are larger in size and have higher metabolic and enzyme activities than the former (Kellogg and Deming, 2014; Mével et al., 2008; Murrell et al., 1999; Simon et al., 2002). Based on these characteristics, we hyp that the differences between the two groups would discriminate their correlations with ARGs. The taxonomic classification for bacterial OTUs is shown in Table S7.

3.4.1. Heat map analysis at the phylum level

The numbers of free-living bacteria in the samples varied from 377 to 20,530 reads (5234 ± 5185 reads), with 17 phyla. The most abundant bacteria at the phylum level were *Proteobacteria* (48.8%), followed by *Bacteroidetes* (25.3%) and *Cyanobacteria* (6.63%), with the remaining bacteria comprising 5%. We found no significant correlation ($p > 0.05$) between total ARGs and free-living bacteria; however, *Margulisbacteria* and unknown phyla were positively correlated with *tet*D, and *Marinimicrobia* was correlated with *tetZ* (Fig. 5a). Meanwhile, particle-attached bacteria in seawater were present at an average of 2553 ± 1643 reads, 3.82–933-fold less than the free-living bacteria. The most abundant phylum was *Proteobacteria* (43.3%), followed by *Bacteroidetes* (24.1%), which were not significantly correlated with ARGs ($p > 0.05$) (Fig. 5b). Instead, *Bdellovibrionota* ($r = 0.39$, $p < 0.05$), *Cyanobacteria* ($r = 0.40$, $p < 0.05$), and *Margulisbacteria*

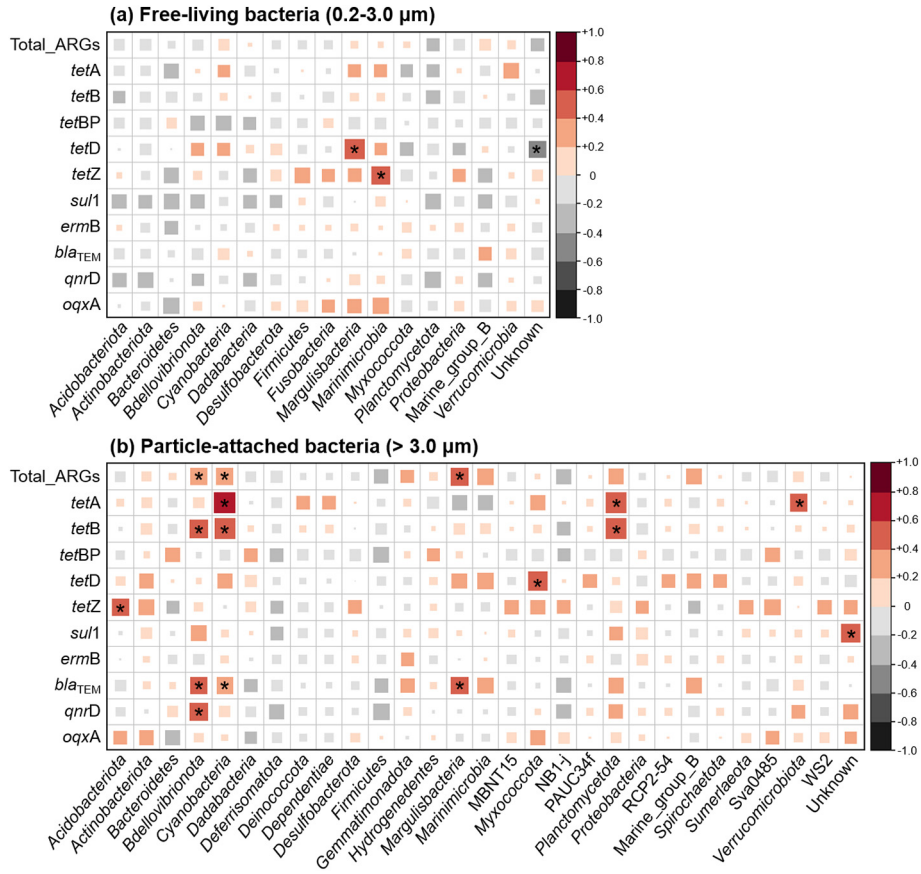

Fig. 5. Heat maps showing the correlations between ARG and bacteria phyla: (a) free-living and (b) particle-attached bacteria. The squares denote the absolute value of correlation coefficients. The correction coefficients are indicated by the degree of colors in the color bar, where red indicates negative correlation coefficients and blue indicates positive correlation coefficients. Regardless of color, the areas of the squares indicate the absolute value of the correlation coefficients, and * indicates significant difference ($p < 0.05$).

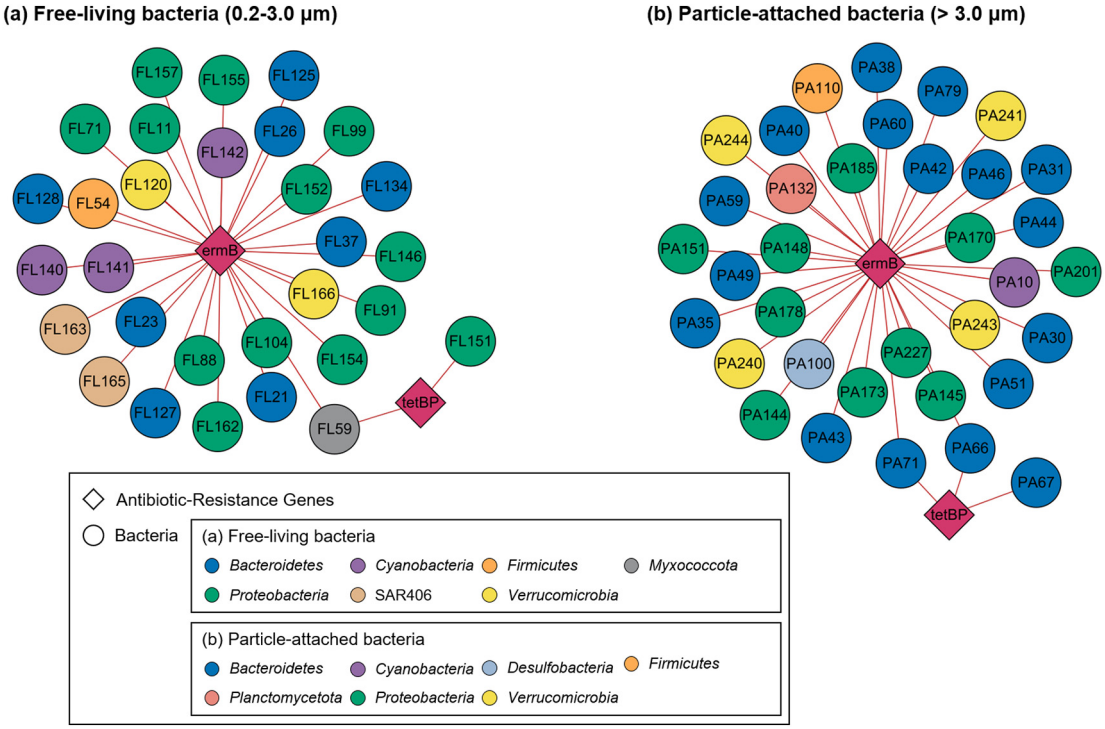

Fig. 6. Correlation networks showing co-occurrence interactions between ARGs and bacterial communities. In the network, nodes were composed of ARGs and bacteria at a genus level, and nodes were connected by edges. The edges were proportional to the LSA values. The numbers indicate OTUs level of free-living (FL) and particle-attached (PA) bacteria, and their taxonomic information are shown in Table S7.

($r = 0.43, p < 0.05$), included in the remainder phyla being comprising less than 5% of abundance, were significantly correlated with total ARGs. Moreover, individual ARGs (*tet*A, *tet*B, *tet*D, bla_{TEM}, and *qnr*D) were also significantly correlated with *Bdellovibrionota.* A previous study reported that *Cyanobacteria* species were dominant in the summer in the Atlantic Ocean (Giovannoni and Vergin, 2012). In this study, *Cyanobacteria* species were less abundant than other bacteria (3.27%) in the western Pacific and Southern Oceans; however, they were highly correlated with ARGs. Therefore, in this study, *Bdellovibrionota*, *Cyanobacteria*, and *Margulisbacteria* could be the potential pathogens carrying the observed ARGs in the ocean.

3.4.2. Network analysis at the genus level

In the free-living bacteria network, 32 nodes and 31 edges were represented, with 166 OTUs at the genus level (Fig. 6a). The network had 30 OTUs belonging to seven phyla (*Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, *Myxococcota*, *Proteobacteria*, SAR406, and *Verrucomicrobia*) affecting ARG abundance. The particle-attached bacteria showed a relatively more complex network (37 nodes and 37 edges) than did the free-living bacteria network (Fig. 6b). The particle-attached bacteria network contained 35 OTUs at the genus level, belonging to 10 phyla (*Bacteroidetes*, *Cyanobacteria*, *Desulfobacterota*, *Firmicutes*, *Planctomycetota*, *Proteobacteria*, and *Verrucomicrobia*). The OTU numbers for the particle-attached bacteria were approximately 1.2-fold greater than those of the free-living bacteria in the networks. These data suggest that the particle-attached bacteria group contained more bacteria communities correlated with ARGs occurrence than the free-living bacterial group. Both *Bacteroidetes* and *Proteobacteria* were predominant phyla in both particle-attached and free-living bacteria groups, which are widely distributed in oceans regardless of latitude (Sul et al., 2013). The abundance of OTUs belonging to *Bacteroidetes* in particle-attached bacteria, accounting for 46%, was 1.8-fold higher than that of free-living bacteria. However, the number of OTUs belonging to *Proteobacteria* in the free-living bacterial network was 1.6-fold larger than that of the particle-attached bacteria, accounting for 42% of free-living bacteria. Meanwhile, *Myxococcota* and SAR406 were bacterial phyla that were present only in the free-living bacteria network, whereas *Desulfobacterota* and *Planctomycetota* were bacterial phyla that were present only in the particle-attached bacteria network. Comprehensively, the network analysis indicated that bacteria potentially carrying ARGs differed between the particle-attached and free-living bacteria groups.

We observed a difference between results of the Pearson's correlation and the network analysis via LSA. For example, *Cyanobacteria*, which showed high correlations between ARGs and particle-attached bacteria (Fig. 5b), included less than three OTUs in each of both bacterial networks *Cyanobacteria* (FL140, FL141, FL142, and PA10) (Fig. 6). In contrast, the correlations between ARGs and *Cyanobacteria* or *Planctomycetota*, which were prominent in the correlation results, would have been partially omitted in the network analysis. The difference between Pearson's correlation and network analysis via LSA was also reported in a previous study that compared microbiological community datasets (Xia et al., 2011). Those authors found missing correlations in both approaches, suggesting that both methods should be utilized in a complementary approach.

The 2 ARG subtypes, *erm*B and *tet*BP showed the most significant number of associations (30 free-living and 35 particle-attached bacteria) (Fig. 6). In previous studies, *erm*B has been detected in sewage and WWTPs (Guan et al., 2018; Rodriguez-Mozaz et al., 2015; Su et al., 2017; Subirats et al., 2017), and the *tet* gene is typically associated with human activities (Baron et al., 2018; Chopra and Roberts, 2001; Daghrir and Drogui, 2013). However, interestingly *erm*B and *tet*BP were not abundant (7.6 ± 4.7% and 0.013 ± 0.011% of the total ARGs, respectively) in this study. Accordingly, the correlation of low content ARGs and various bacterial species suggests the vulnerability of bacteria to dissemination of these ARGs. Moreover, *tet*BP was positively correlated ($p < 0.01$) with the *intI*1 gene (Table S5). This suggests that *tet*BP is more likely to be transferred horizontally among bacteria in marine environments (Zhang et al., 2017), while *erm*B may be transferred via other mechanisms. Further research is required to better understand which mechanisms underly the occurrence and dissemination of *erm*B between bacteria. In contrast, no free-living or particle-attached bacteria correlated with bla_{TEM}, which accounted for the largest proportion of total ARGs in most sampling locations, were detected

in this study. Meanwhile, several bacteria were positively correlated with both *erm*B and *tet*BP as follows: *Myxococcota* (FL59) and *Bacteroidetes* (PA66, and PA71) for the particle-attached bacteria. This result suggests that FL59 and PA71 may carry both *erm*B and *tet*BP.

4. Conclusions

This study investigated the prevalence and abundance of ARGs and bacterial communities along a transect from the western Pacific Ocean (36°N) to the Southern Ocean (74°S). The following conclusions were drawn from our analyses:

- The relative abundance of total ARGs detected along the transect from the western Pacific Ocean to the Southern Ocean was equivalent to the level previously demonstrated in clean aquatic environments, such as drinking water.
- The absolute total ARG abundance in the western Pacific Ocean was approximately 1.8-fold higher than in the Southern Ocean. Moreover, ARGs in the Southern Ocean were less abundant than in the western Pacific Ocean, but increased toward Antarctica, possibly due to natural terrestrial sources or human activities at research stations.
- *β*-Lactam and tetracycline resistance genes were dominant in all samples (88–99%), indicating that they are likely the key ARGs in the ocean.
- In the particle-attached bacteria group (>3.0 μm), *Bdellovibrionota*, *Bacteroidetes*, *Cyanobacteria*, *Margulisbacteria*, and *Proteobacteria* were associated with ARGs. In the free-living bacteria group (0.2–3.0 μm), *Bacteroidetes* and *Proteobacteria* were correlated with ARGs. Therefore, these bacteria are candidates for carrying the observed ARGs in the ocean.

We detected ARGs in the Southern Ocean, where little information on antibiotics or ARGs is available, suggesting that antibiotic resistance is threatening polar regions considered to be pristine. Our data suggest that controlling pollution and preventing potential threats of ARGs to the marine environment are urgent. Our results allow targeting and controlling ARGs and associated bacteria according to latitude, thus reducing potential risks in the future. Moreover, while most ARG studies are still being conducted locally, this study is significant in its demonstration of their presence on a global scale. To better understand ARG distribution, long-term monitoring of their variations and bacterial communities in the open ocean system on a global scale is required. Further metagenomic studies are required in the future, since there are many more types of ARGs in the oceanic environment than those detected in this study.

CRediT authorship contribution statement

Jiyi Jang: Investigation, Visualization, Writing - original draft. **Jiyeon Park**: Conceptualization, Writing - review & editing, Supervision. **Chung Yeon Hwang**: Methodology, Writing - review & editing. **Jinhee Choi**: Investigation. **Jingyeong Shin**: Investigation. **Young Mo Kim**: Methodology, Writing - review & editing. **Kyung Hwa Cho**: Writing - review & editing. **Jung-Hyun Kim**: Writing - review & editing. **Yung Mi Lee**: Writing - review & editing. **Bang Yong Lee**: Project administration, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.153360.

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