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Diverse but low abundance resistome and virulome in Fildes Peninsula intertidal sediments

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Abstract The Fildes Peninsula is among the Antarctic regions most affected by human activity. As the interface between terrestrial and marine ecosystems, the intertidal is a gateway for ecological and public health risks. Antibiotic resistance genes (ARGs) and virulence factor genes (VFGs) intensify these risks by transferring pathogenic traits via horizontal gene transfer. However, a comprehensive assessment of ARGs and VFGs in Fildes Peninsula intertidal sediments is lacking. We addressed this gap by performing shotgun metagenomic sequencing on 14 intertidal sediments. We detected 20 ARG types comprising 397 subtypes. Multidrug, bacitracin, and polymyxin resistance dominated the resistome, although their overall abundance was relatively low. A total of 4,204 VFGs were identified and were primarily associated with adherence, motility, immune modulation, and effector delivery. The abundance of VFGs was also relatively low and significantly correlated with the abundance of ARGs. Additionally, we also identified 787 mobile genetic elements (MGEs) that carried 138 VFGs but found no ARGs associated with MGEs at the contig level. Host assignment implicated taxa such as *Ilumatobacter* as major ARG carriers and detected opportunistic pathogen *Pseudomonas aeruginosa* carrying *CAM-1*, a metallo-β-lactamase gene. In summary, our results reveal a highly diverse but generally low-abundance resistome and virulome in Fildes Peninsula intertidal sediments, with limited evidence for MGE-mediated ARG dissemination. However, they emphasize the necessity of monitoring to protect Antarctic microbial integrity.

Keywords Antarctic, intertidal sediments, antibiotic resistance genes (ARGs), virulence factor genes (VFGs), metagenomic sequencing, mobile genetic elements (MGEs)

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1 Introduction

The increasing prevalence of antibiotic resistance genes (ARGs) poses a significant threat to global public health. Despite its remoteness, ARGs have been detected in various Antarctic habitats, including soils, lakes, glaciers, and animal feces, indicating their widespread presence in this remote environment (Segawa et al., 2024; Tallada et al., 2022; Thajudeen et al., 2025; Wang et al., 2016; Yuan et al., 2019). Polar soil ARGs exhibit a distinct phylogenetic clustering, suggesting a unique evolutionary origin potentially driven by the extreme environmental conditions of polar environments (Thajudeen et al., 2025; Van Goethem et al., 2018). Furthermore, the distribution of ARGs is influenced by factors such as human activity (Wang et al., 2016), soil characteristics (Wang et al., 2016), and the presence of animals (Segawa et al., 2024).

Virulence factor genes (VFGs) are found in pathogenic microorganisms, enhancing their infectivity and pathogenicity. They are crucial for the transmission of infection and the development of disease, providing molecular markers for monitoring potential pathogens in the environment (Liu et al., 2022). Some pathogenic bacteria can carry both VFGs and ARGs, which enhance their environmental adaptation (Tigabu et al., 2025) and promote antibiotic resistance (Derakhshan et al., 2021), thereby increasing the difficulty of therapeutic management. Therefore, monitoring the co-existence of VFGs and ARGs helps assess the potential threat posed by pathogens within the environment and facilitates the development of effective control and management strategies.

Studies have identified a significant presence of ARGs in Antarctic soils and sediments. For instance, research on Deception Island’s soil metagenomes revealed ARGs associated with antibiotic efflux, inactivation, and target alteration, with *Pseudomonas* and *Psychrobacter* being key taxa (Santos et al., 2022). Many ARGs are naturally occurring, produced by antibiotic-producing bacteria (Van Goethem et al., 2018), or perform ecological roles such as cellular signaling (Andersson and Hughes, 2014) and detoxification (Zhao et al., 2024). The geographical isolation and unique evolutionary pressure in Antarctica have led to substantial ARG sequence novelty (Van Goethem et al., 2018). In addition to ARGs native to Antarctica, migrating birds and penguins can act as vectors that transport and/or enrich ARGs in Antarctic environments, highlighting the role of wildlife in ARG dissemination (Zhou et al., 2024). Moreover, long-range atmospheric transport and human activities, such as those from ships, research stations, and tourists, contribute to the introduction of ARGs in coastal and inland Antarctic sites (Wang et al., 2016). These trends highlight the importance of evaluating the distribution of antibiotic resistance in this unique and vulnerable environment.

Despite the Antarctic ecosystem being relatively isolated, the presence of ARGs due to human activities poses a significant risk to both the environment and human health. The potential for ARGs to transfer to human pathogens emphasizes the importance of monitoring and mitigating their spread. Human activities, including the operation of research stations and increased tourism, have been linked to the introduction and spread of ARGs in Antarctica (Depta and Niedźwiedzka-Rystwej, 2023). Areas with higher human activity show a greater diversity and abundance of ARGs compared to more remote regions, suggesting a direct correlation between human presence and ARG prevalence (Jara et al., 2020).

The Fildes Peninsula (62°08′S to 62°14′S and 59°02′W to 58°51′W), located southwest of King George Island, is the largest ice-free area in the South Shetland Islands. Its close proximity to South America, relatively mild climate, and good natural harbors and landing sites make it one of the most active and populated regions of Antarctica, with substantial scientific research and tourism. Furthermore, the peninsula includes one specially protected area (ASPA-125) and supports large seabird and penguin colonies (e.g., Adélie and Gentoo penguins), which concentrate fecal inputs and can locally amplify microbial and ARG loads.

Intertidal zones refer to areas alternately exposed to air and seawater by tidal action, serving as critical interfaces between terrestrial and marine ecosystems. These zones are important regions for the exchange and transfer of microbes and ARGs, with ARGs present in marine sediments and waters that can be taken up by coastal fauna, transported ashore by birds, or introduced to humans through shore-based activities, posing zoonotic and public health risks (Gabashvili et al., 2022). Climate warming and glacier melt further mobilize ARGs from terrestrial ice and permafrost into coastal sediments, increasing the potential for ARG dissemination in Antarctic intertidal zones (Zhu et al., 2017). Although a few studies have characterized ARGs and ARG-carrying bacteria on the Fildes Peninsula, the intertidal zone remains poorly investigated. A comprehensive understanding of ARGs and ARG-carrying pathogens in this ecologically sensitive area is still missing, highlighting the need for integrated studies using genomics and large-scale data analytics.

To address this gap, we conducted a metagenomic sequencing study on surface sediments collected from intertidal zones of the Fildes Peninsula, profiling the distribution of ARGs and VFGs as well as their co-occurrence with mobile genetic elements (MGEs). Our work provides the first comprehensive report on ARGs and associated virulence determinants in the Fildes Peninsula intertidal zone, filling a major knowledge gap about ecological biohazards in this important Antarctic region.

2 Materials and methods

2.1 Sample collection

Intertidal sediment samples from 0 to 5 cm depth were collected around Fildes Peninsula in 2020 during the 36th Chinese Antarctic Research Expedition (Figure 1). A total of 14 intertidal sediment samples were collected, covering both the eastern and western coasts of the Fildes Peninsula. The samples were collected using sterilized shovels and stored in sterilized plastic bags, while wearing polyethylene gloves to avoid contamination. The collected sediment samples were stored at −80 ℃ until transported to the Polar Research Institute of China in Shanghai for DNA extraction.

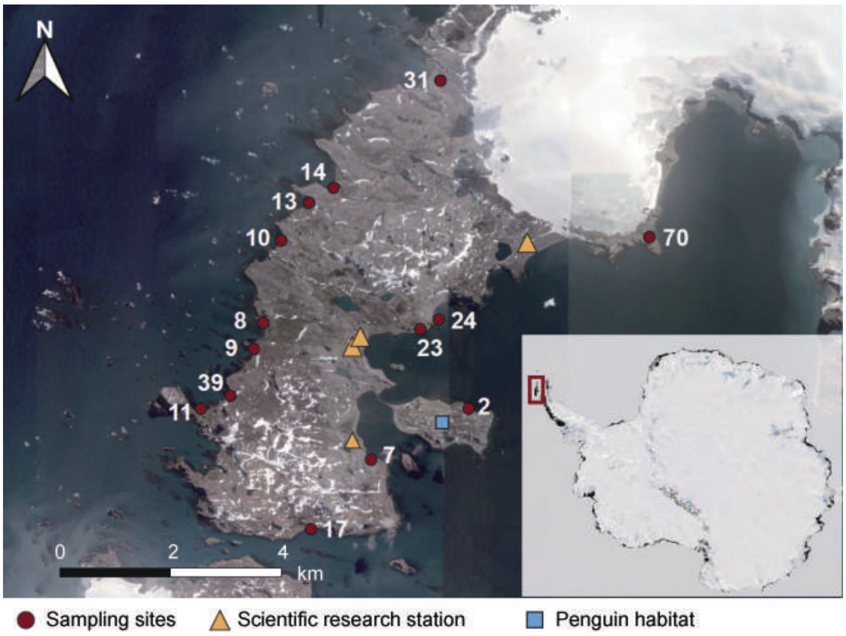

Figure 1 Map of sampling sites for intertidal sediments in the Fildes Peninsula, with the location of the research station and penguin colonies being marked. Sampling sites 7, 23, and 24 are situated approximately 370 m, 1,230 m, and 1,577 m from the research station, respectively.

2.2 DNA extraction and metagenome sequencing

Total DNA was extracted from sediment samples using the modified SDS-based method described by Natarajan et al. (2016). Briefly, 2 g of sediment was added to a 15 mL centrifuge tube containing glass beads, the extraction buffer, lysozyme, and proteinase K. The samples were homogenized and incubated for 30 min at 37 ℃ in a water bath. After incubation, samples were homogenized twice as before, followed by the addition of 20% SDS and incubation at 55–60 ℃. After centrifugation at 10,000×g, the supernatant was collected. Then, 5 mL PCI (Phenol-Chloroform-Isoamyl Alcohol) was added, and the mixture was centrifuged at 12,000×g. The supernatant was collected and added to sodium acetate and isopropyl alcohol, then incubated at 4 ℃ overnight. After centrifugation at 12,000×g, the supernatant was carefully removed to prevent DNA loss. The resulting DNA pellet was then washed with pre-cooled 70% ethanol and resuspended in 50 μL TE buffer. Specific details can be found in the supplementary (Text S1). A total of 14 sediment DNA samples were sent to Biozeron (Shanghai, China) for metagenomic sequencing on an Illumina NovaSeq platform using the PE150 sequencing strategy.

2.3 Metagenomic data analysis

The sequencing reads were first quality filtered using fastp (v0.22) by removing any reads containing >30% of low-quality bases and length <75 bp (Chen et al., 2018). The quality-filtered reads were assembled using Megahit (v2.15) (Li et al., 2015) with default k-mers, retaining only assembled contigs >500 bp. The open reading frame prediction was performed using Prodigal (v2.6.3) (Hyatt et al., 2010). The details of the metagenomic assembly are presented in Table S1.

2.4 ARGs, VFGs, and MGEs prediction and annotation

ARGs annotation was performed using ARGs-OAP (Yin et al., 2018) using the Structured Antibiotic Resistance Gene (SARG) Database (v3.2.1) through a two-stage process. In the pre-screening stage, ARGs and 16S rRNA genes were identified using the built-in database within ARGs-OAP and a Perl script. In the second stage, the identified ARG sequences were aligned against the SARG reference database. Alignment parameters were set as follows: identity >80%, *e*-value cutoff $<1\times10^{-5}$, and alignment length >25 bp (Thajudeen et al., 2025). Subsequently, normalization was performed using the following formula.

$$\text{Abundance}=\sum_{1}^{n}\frac{N_{\text{ARG-like sequence}}\times L_{\text{reads}}/L_{\text{ARG reference sequence}}}{N_{\text{16S sequence}}\times L_{\text{reads}}/L_{\text{16S sequence}}}$$

where $N_{\text{ARG-like sequence}}$ is the number of the ARG-like sequences annotated to one specific ARG reference sequence; L_{reads} represents the length of the reads; $L_{\text{ARG reference sequence}}$ is the nucleotide sequence length of the corresponding specific ARG reference sequence; $N_{\text{16S sequence}}$ is the number of the 16S rRNA gene sequences; $L_{\text{16S sequence}}$ is the full length of the 16S rRNA gene. n is the number of mapped ARG reference sequences belonging to the ARG type or subtype.

The abundance of the ARG was normalized against the copy of the 16S rRNA gene at both type and subtype levels. VFGs were annotated by querying the predicted amino acid sequences against the Virulence Factor Database (VFDB 2025, downloaded on 19 July 2025) (Liu et al., 2022) using ARGs-OAP with clean reads, applying an *e*-value threshold of 1×10^{-5}, identity >70%, and an alignment length >50 bp as described previously (Kim et al., 2022). The identity of ARGs and VFGs is calculated by querying the predicted amino acid sequences against the Antibiotic Resistance Gene Database (v3.2.1) and Virulence Factor Database (VFDB 2025), respectively.

MGEs were classified and quantified by aligning sequences against the MGE Database (Pärnänen et al., 2018) using ARGs-OAP with clean reads, applying identity ⩾ 80% and coverage ⩾ 70% (Ke et al., 2023a). We further explored, the coexistence of ARGs and VFGs with MGEs at the contig level, including plasmids, insertion sequences (ISs), and viruses. PlasmidFinder (v2.1.6) (Carattoli et al., 2014) and ISfinder (Siguier, 2006) were used to predict whether the ARG- or VFG-carrying contigs are of plasmid origin or flanked by ISs, respectively. Additionally, VirSorter2 (v2.2.3) (Guo et al., 2021) and CheckV (v1.0.1) (Nayfach et al., 2021) were used to identify contigs of viral origin that carried ARGs or VFGs. The host of ARGs and exotoxin-type VFGs were identified at the contig level (Ke et al., 2023b) by using MEGAN (v6.25.10) against the NR Database.

2.5 Plotting and statistical analysis

The pie charts and stacked bar charts were generated using the ggplot2 package in R (v4.3.0). The heatmap of the top 50 ARG subtypes by abundance was produced using the pheatmap package. Chord diagrams were generated using the circlize package to visualize the hosts of ARGs and exotoxin. Correlations between the abundance of ARGs and VFGs, ARGs and MGEs, and VFGs and MGEs were calculated and visualized using the ggpmisc and ggpubr packages. Additionally, we employed FDR (False Discovery Rate) to adjust the raw *p*-values from the correlation tests, using the "p.adjust" function in R (v4.3.0) for the calculations.

3 Results

3.1 The relative abundance and composition of antibiotic resistance genes

A total of 1,493 ARGs were identified, and the average sequence identity of these ARGs was 84.62%±0.70%, with sites 23 and 24 exhibiting higher identity compared to other sites (Figure S1). These ARGs were classified into 20 ARG types, with multidrug (37.05%) and bacitracin resistance (26.35%) being the most abundant (Figure 2a). Furthermore, the abundance of multidrug resistant ARGs were significantly higher than that of polymyxin (16.86%), mupirocin (4.29%), novobiocin (3.57%), and beta-lactam (3.01%) resistant ARGs (Kruskal-Wallis test, all $p<0.0001$). The normalized abundance of ARGs ranged from 4.6×10^{-2} to 9.8×10^{-2} copies of ARGs per copy of the 16S rRNA gene, with higher abundance at sites 17, 23, and 24 on the east coast (Figure S2a). However, there was no significant difference in ARGs abundance between the east and west coasts (Figure S2b) (Kruskal-Wallis test, $p=0.0707$).

More than 58% of the resistome abundance was attributed to the mechanism of antibiotic target alteration (Figure 2b), conferring resistance against bacitracin, vancomycin, and polymyxin. Efflux pump accounted for 30.09% of ARGs, mainly associated with multidrug resistance and resistance against tetracycline and macrolide-lincosamide-streptogramin. Enzymatic inactivation (8.08%) was primarily attributed to ARGs against beta-lactam and aminoglycoside. Additionally, 3.20% of ARGs were attributed to antibiotic target replacement and antibiotic target protection, which confer resistance to antibiotics, including trimethoprim, quinolone, and tetracycline.

A total of 397 ARG subtypes were identified, with ARGs against beta-lactam having the most subtypes (123 subtypes), followed by those with multidrug resistance (77 subtypes). The top 50 most abundant ARG subtypes accounted for 93.6% of all ARGs (Figure 2c), with the highest number of subtypes associated with multidrug resistance (20 subtypes). Among these, *bacA* (against bacitracin) was the most abundant, followed by *RanA* (multidrug resistance) and *ugd* (against polymyxin); their abundances were 2.2×10^{-1}, 1.7×10^{-1}, and 8.3×10^{-2} copies of ARGs per copy of 16S rRNA gene, respectively.

3.2 The relative abundance and composition of virulence factor genes

A total of 4,204 VFGs were identified. The average sequence identity was 84.64%±0.86%, with sites 23 and 24 exhibiting higher identity compared to other sites (Figure S3). The identified VFGs were mainly classified under motility (18.91%) (Figure 3a), whereas adherence was the most abundant category (25.59%). The abundance of adherence was significantly higher than that of regulation (Kruskal-Wallis test, $p=0.0115$), which was the second most abundant VFG (14.52%). Followed by the motility (14.50%), nutritional/metabolic factor (11.54%), stress survival (10.66%), effector delivery system (8.70%), and biofilm formation (7.18%) (Figure 3b). The relevant definitions for these VFGs were shown in Table S2. The normalized abundance of VFGs in different samples ranged

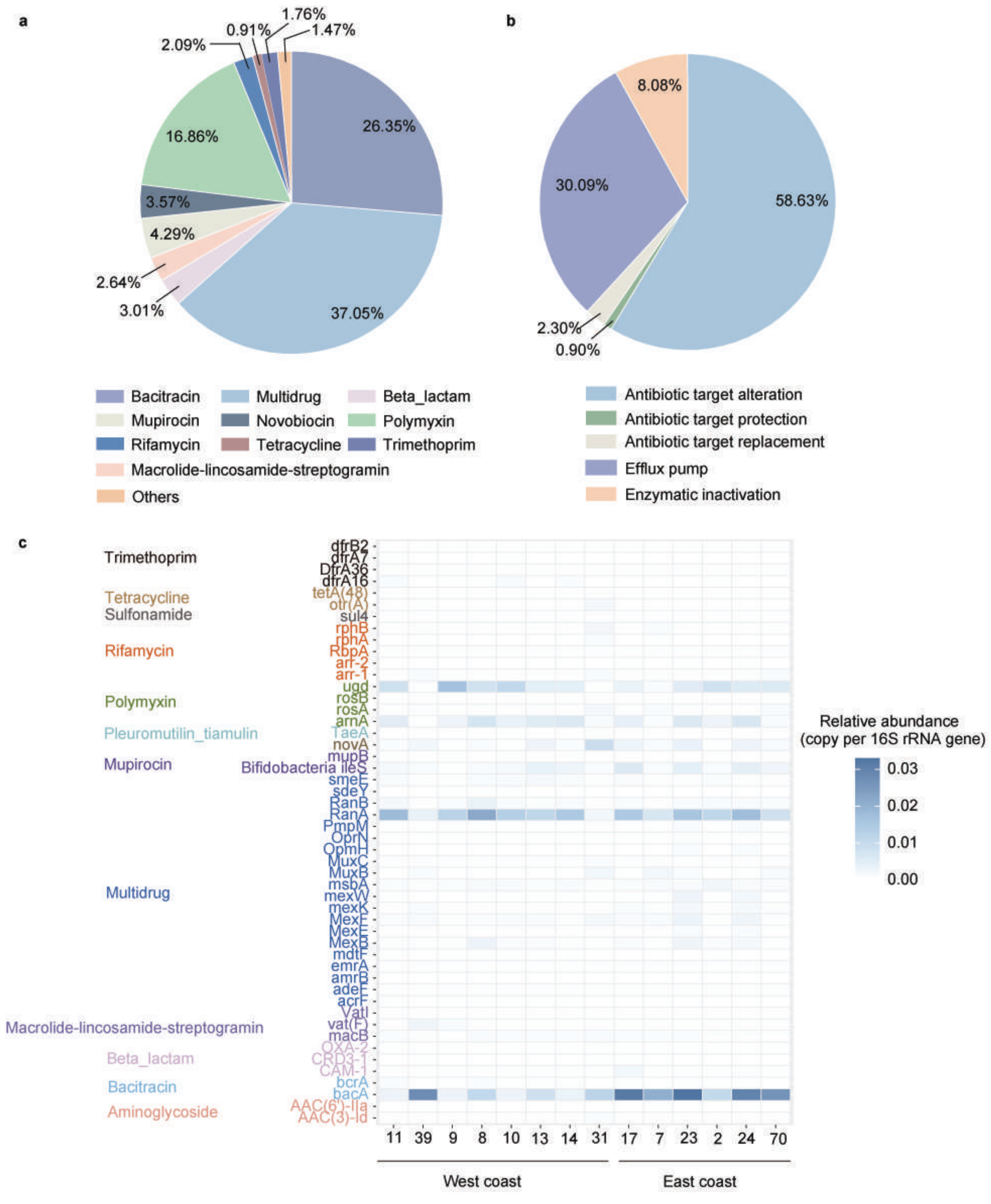

Figure 2 he relative abundance and the composition of Antibiotic Resistance Genes (ARGs). **a**, composition of ARGs across all samples; **b**, composition of resistance mechanisms across all samples; **c**, heatmap showing the abundances of the top 50 most abundant ARG subtypes. Diverse ARGs are identified in intertidal sediments of the Fildes Peninsula, with *RanA* and *bacA* being the most dominant ARGs.

from 4.8×10^{-2} to 3.9×10^{-1} copies of VFGs per copy of the 16S rRNA gene, with higher relative abundance at sites 17, 23, and 24 also observed (Figure 3c). Additionally, the VFGs abundance between the east and west coasts was not significantly different (Figure 3d) (Kruskal-Wallis test, p=0.0707). In addition, the abundance of VFGs was significantly correlated with the ARGs (Spearman correlation, p<0.001, R^2=0.68) (Figure S4).

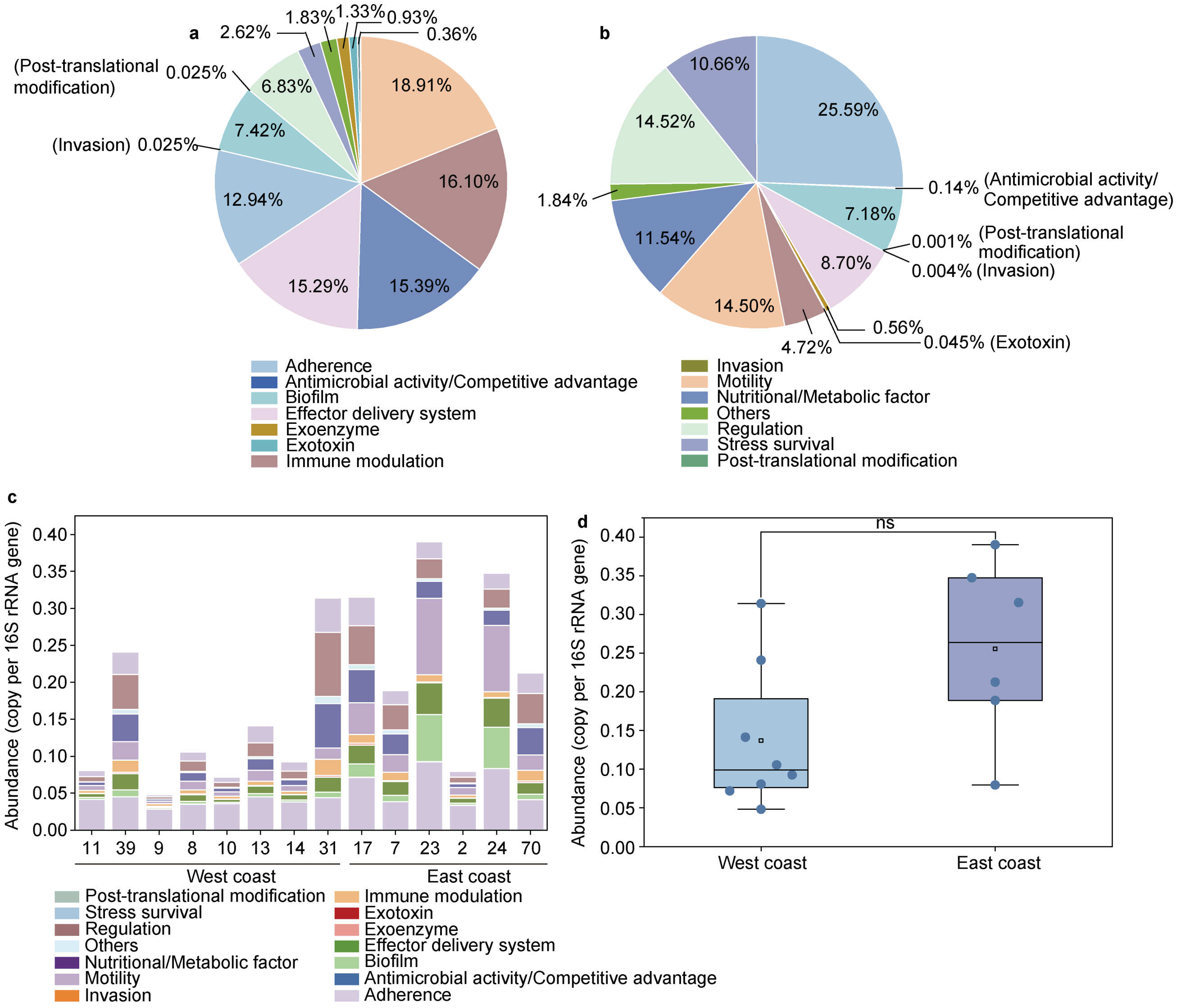

Figure 3 The relative abundance and the composition of virulence factor genes (VFGs). **a**, the diversity of VFGs across all samples; **b**, composition of VFGs across all samples; **c**, the abundance of VFGs in different samples; **d**, comparison of VFGs abundance between the west coast and east coast. Diverse VFGs are presented across the intertidal sediments of the Fildes Peninsula, exhibiting uneven distribution across the sampling sites.

3.3 Mobile Genetic Elements (MGEs) composition and coexistence with ARGs and VFGs

A total of 787 MGEs were identified by aligning predicted protein sequences against the MGE Database (Pärnänen et al., 2018). These MGEs were classified into 24 types, mainly including insertion element *IS91*, *transposase*, *tniA*, *tniB*, integrase, *ISRj1*, *ist* (*istA*, *istB*) (Figure 4a). The abundance of MGEs per sample ranged from 2.7×10^{-2} to 2.0×10^{-1} copies of MGEs per copy of 16S rRNA gene, exhibiting significant correlation with the relative abundance of ARGs (Spearman correlation, $p<0.001$, $R^2=0.85$) and VFGs (Spearman correlation, $p<0.001$, $R^2=0.82$) (Figure S5).

We then further explored the co-occurrence of ARGs and VFGs with MGEs, including ISs, viruses, and plasmids at the contig level. Analysis identified 64 VFGs carried by viruses, which were classified as Caudoviricetes (Figure 4b). Virus-carried VFGs were mostly classified under the categories of effector delivery systems (62.5%) and immune modulation functions (34.4%). Furthermore, 14 VFGs were carried by insertion sequence and were mainly attributed to motility functions (45.5%) and nutritional/metabolic factors (22.6%). In addition, 60 VFGs were carried by integrase, and all of these VFGs were classified as adherence functions. However, we did not identify any ARG being carried by MGEs.

3.4 Hosts of ARGs and VFGs

Hosts of ARGs were identified at the contig level. In

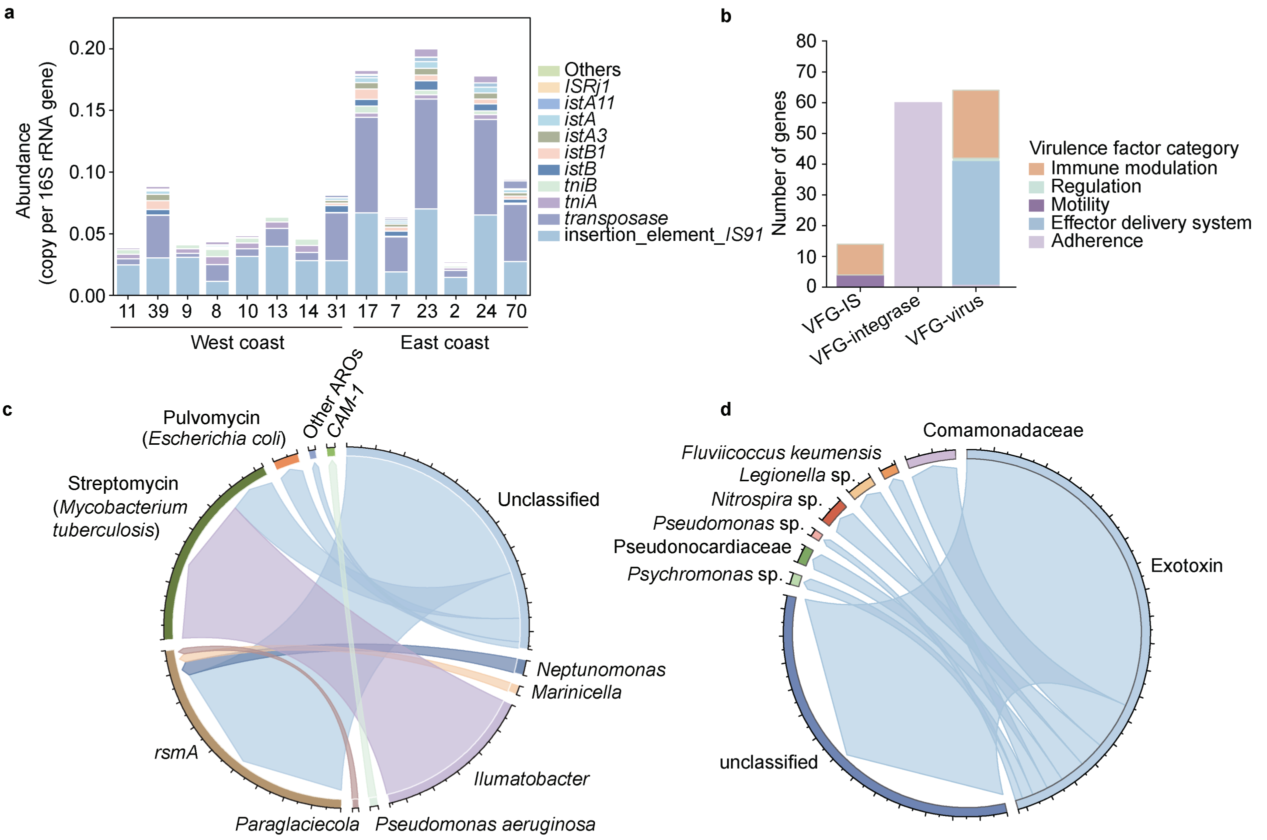

Figure 4 The composition of Mobile Genetic Elements (MGEs) and the coexistence of ARGs and VFGs with MGEs. **a**, the abundance and composition of MGEs in each sample; **b**, the coexistence of MGEs with ARGs and VFGs; **c**, the hosts of ARGs; **d**, the hosts of exotoxin. Integrases and viruses are the main MGEs carrying VFGs, while no coexistence between ARGs and MGEs has been identified. The hosts of exotoxin were mainly *Legionella* sp. and *Nitrospira* sp.

addition to unclassified bacteria, 5 additional ARG hosts were identified, including *Neptunomonas*, *Marinicella*, *Ilumatobacter*, *Paraglaciecola*, and *Pseudomonas aeruginosa* (Figure 4c). *Ilumatobacter* had the highest relative abundance of 31.9% across all hosts except for the unclassified host, and it was the primary host for the aminoglycoside antibiotic (*Mtub_rpsL_STR*). *Pseudomonas aeruginosa* was the host for cephalosporin, penicillin beta-lactam, carbapenem (*CAM-1*), while other bacteria were carriers of the phenicol antibiotic, diaminopyrimidine antibiotic, and fluoroquinolone antibiotic (*rsmA*). In addition, as exotoxins are VFGs that can be released into the environment, their hosts were also identified. We identified multiple species that can release exotoxins, including *Psychromonas* sp., *Nitrospira* sp., *Legionella* sp., and *Fluviicoccus keumensis*, among others (Figure 4d).

4 Discussion

In this study, a shotgun metagenomic sequencing approach was used to identify ARGs, VFGs, and MGEs in intertidal sediments from the Fildes Peninsula. Our analysis revealed that the intertidal zone harbors abundant ARGs and VFGs but exhibits a low dissemination risk. These genes are likely the product of natural evolution by microorganisms to adapt to low-temperature and oligotrophic environments, rather than originating from external contamination (Van Goethem et al., 2018). With increasing human activities on the Fildes Peninsula, there is a need to monitor potential ecological health risks within the intertidal environments.

Our results revealed diverse but low-abundance ARGs in the intertidal zone of the Fildes Peninsula. The 397 ARG subtypes across 20 ARG types identified were even more diverse than that reported for drinking water distribution systems (213 ARG subtypes) (Ke et al., 2023a), prairie soils (268 ARG subtypes) (Qian et al., 2021), and other Antarctic soils and waters (154 and 114 ARG subtypes, respectively) (Thajudeen et al., 2025; Zhang et al., 2022). This high diversity, together with the overall low abundance, suggests that these ARGs are most likely intrinsic (Van Goethem et al., 2018)—arising from microbial adaptation to extreme and variable environmental conditions rather than recent anthropogenic inputs. Additionally, most of the ARG sequences exhibited less than 90% homology with known sequences in the database (Figure S1). Identity is a crucial measure of sequence similarity, and higher sequence identity typically implies that two sequences share a common evolutionary origin (Joshi and Xu, 2007). While the observed low sequence identity aligns with the long-term evolution of native microorganisms, alternative explanations must also be considered. The incompleteness of current reference databases for environmental microbes and differences in sampling and analytical approaches among studies could also contribute to the observed low identity patterns. Therefore, the sequence identity should be interpreted as supporting, rather than definitive, evidence for an intrinsic origin.

The abundances of VFG and ARG were distributed highly unevenly among the sampling sites (Figures S2 and 3c). For instance, sites 23 and 24, which are located close to a research station, exhibited high levels of ARGs and VFGs; site 7, which is even closer to a research station, exhibited only moderate levels of both ARGs and VFGs; site 2, which is near penguin colonies, had low levels of both ARGs and VFGs. The observed spatial heterogeneity in ARG and VFG abundances likely reflects a combination of source distribution and environmental retention rather than simple distance from human and penguin activities. Anthropogenic inputs from the research station and its wastewater are recognized as key sources of resistance determinants in Antarctic coastal environments (Clark et al., 2025; Jara et al., 2020), but variable sewage-treatment performance and episodic wastewater releases can discharge ARG- and VFG-bearing bacteria that travel along specific pathways and are deposited downstream. Thus, sites 23 and 24 may lie in retention zones and accumulate higher loads, while site 7—despite being closer in straight-line distance—may fall outside the dominant plume trajectory and therefore show only moderate signals. Conversely, the low ARG/VFG levels at site 2 near penguin colonies suggest that proximity to penguin colonies does not necessarily elevate ARG/VFG abundances. Reports of penguin activity enriching ARGs in lake sediment are based on analyses of ornithogenic guano sediments (Zhou et al., 2024) and intestinal tract samples (Gutiérrez et al., 2024). Thus, spatially localized input may be insufficient to affect nearby sampling sites. In addition, metagenomic methods detect both live cells and extracellular (relic) DNA, so elevated ARG signals at some sites may reflect historical contamination rather than ongoing inputs of viable bacteria. Resolving these alternatives will require hydrodynamic modeling and RNA-based analyses to determine whether plume routing, sedimentary retention, wildlife contributions, or legacy DNA account for the observed spatially uneven resistome and virulome. The presence of ARG only indicates the potential of antibiotic resistance, whose phenotype depends on factors such as gene expression and environmental conditions (Deekshit and Srikumar, 2022). These findings suggest that while human and penguin activities may increase the abundance of antibiotic-resistant bacteria, the genes conferring resistance can persist at significant levels in remote places, even in the absence of human or animal intervention. The latter could be driven by the harsh environmental conditions (Lou et al., 2023; Van Goethem et al., 2018), with the interplay between antibiotic-resistant phenotypes and the presence of ARG warranting further studies in natural environments.

Multidrug resistance was the most abundant ARG type in our samples (Figure 2), echoing findings from many other studies (Jiang et al., 2024; Ke et al., 2023a) and reflecting microbes' tendency to favor broadly effective survival strategies under stress (Chen et al., 2016; Wright, 2007). At the subtype level, *bacA* (bacitracin) dominated the intertidal sediments, a pattern also observed in polar glacier forelands (Thajudeen et al., 2025), the Qinghai-Tibet Plateau (Chen et al., 2016), and the Yangtze River (Jiang et al., 2024). The *bacA* gene is linked to undecaprenyl pyrophosphate metabolism and cell wall biosynthesis (El Ghachi et al., 2004), which may be favored in intertidal microbes because maintaining cell wall integrity helps resist osmotic and salinity fluctuations (Jiang et al., 2024). Consequently, antibiotic target alteration emerged as the primary resistance mechanism in our intertidal samples, in contrast to efflux pump-dominated mechanisms reported in glacier forelands (Thajudeen et al., 2025). Because target alteration is typically narrow and specific (Abbas et al., 2024), while efflux pumps confer broad specificity (Nikaido and Pagès, 2012), these differences imply that local environmental pressures shape not only ARG composition but also the dominant resistance strategies. Additionally, the relative abundance of the multidrug subtype *RanA* in our samples is distinct from the multidrug subtypes dominant in other Antarctic soils and aquatic systems of the Fildes Peninsula (Santos et al., 2022; Zhang et al., 2022). This reflects the heterogeneity in the specific multidrug determinants across nearby microhabitats, suggesting local selection, stochastic colonization, or differential horizontal transfer events shape ARG subtype distributions even within a geographically limited region.

VFGs associated with adherence were the most abundant, with the *GroEL* gene being the most prevalent (21%). *GroEL* is a multifunctional protein that plays an important role in bacterial adaptation to environmental stresses and promotes cell adherence (Zhu et al., 2023). This finding is consistent with previous studies indicating that VFGs often perform multiple functions within cells (Niu et al., 2013). Strikingly, we identified 39 exotoxins, including phospholipase C, hemolysin III/A, non-hemolytic enterotoxin A, and colibactin. These exotoxins might disrupt host cell structure and function by compromising cell membrane integrity (Liu et al., 2025) or inducing DNA damage (Li et al., 2019). The number of exotoxins was greater than in a previous study on the Qinghai-Tibet Plateau (Gou et al., 2025), suggesting that ecological risks in polar regions may warrant further study. Exoenzymes are also important VFGs that promote infection in diverse ways. Eighteen exoenzymes were identified, with the most abundant being Zmp1 (M13 family metallopeptidase) and eno (phosphopyruvate hydratase). These enzymes exert pathogenic effects by degrading key host proteins (Petrera et al., 2012) and promoting adhesion (Setiabudy et al., 2024), respectively. Therefore, these exoenzymes may disrupt intertidal ecosystems by affecting food webs.

The opportunistic pathogen *Pseudomonas aeruginosa* (Hardie Boys and Pletzer, 2025) was detected in intertidal sediments (Figure 4c). This bacterium carries the *CAM-1* gene, which encodes a metallo-β-lactamase capable of hydrolyzing a wide range of antibiotics, including cephalosporins, penicillins, and carbapenems (Kang et al., 2024). Notably, *P. aeruginosa* has also been isolated from Antarctic permafrost (Efimenko et al., 2018). Although no other pathogenic bacteria were identified, this finding suggests a need to focus on *P. aeruginosa* as a potential risk in intertidal ecosystems of Fildes Peninsula. Altogether, the identification of exotoxins, exoenzymes, and pathogenic bacteria revealed multiple potential risks in the intertidal environment, although their currently low abundance suggests a limited transmission risk.

Many studies have demonstrated that horizontal gene transfer (HGT) mediated by MGEs is a significant route for the dissemination of ARGs and VFGs (Adyari et al., 2025; Jiang et al., 2017). In our sediment samples, we detected a variety of MGEs, with those associated with VFGs including ISs, viruses, and integrases (Figure 4). However, they represent only 0.19% of all detected VFGs, a proportion lower than those reported in Inexpressible Island (Dong et al., 2025), indicating that MGE-mediated transfer of VFGs in these sediments is likely limited. Comparatively, we did not detect any MGEs carrying ARGs in the sediments, further suggesting a low potential for MGE-mediated ARG transfer at these sites. This pattern is consistent with findings that MGE-associated ARGs are far more abundant in sewage than in river water or sediments (Adyari et al., 2025), supporting the interpretation that our intertidal sampling area is less exposed to anthropogenic inputs and is relatively pristine. Nevertheless, due to technical constraints including unsaturated sequencing, incomplete metagenomic assembly, short contigs, or novel, uncharacterized MGEs, the potential for ARGs to undergo horizontal gene transfer cannot be eliminated.

Despite the low incidence of ARGs and VFGs on MGEs, we observed positive correlations among the overall abundances of ARGs, VFGs, and MGEs (Figure S5). These correlations are consistent with the co-selection theory, in which shared selective pressures (environmental stressors, residual contaminants, and so on) can favor both resistance and virulence determinants, while HGT can facilitate their dispersal. Such dynamics may promote the emergence of bacterial strains with both increased virulence and resistance, providing a selective advantage in hostile environments (Hussain et al., 2023); this could be relevant for survival in cold, oligotrophic Antarctic conditions. However, although abundance correlations are suggestive, they do not prove co-localization or direct MGE-mediated transfer. We did not find ARGs and MGEs on the same contig in our assembly, which could reflect a genuine absence of co-localization or could result from technical limitations (e.g., incomplete assemblies, short contigs, low sequencing depth, or databases that miss novel MGE sequences). Similar observations have been reported in other relatively pristine environments, such as the Qinghai-Tibet Plateau, where ARGs were rarely found on MGEs (Chen et al., 2016), supporting the idea that horizontal transfer of ARGs may be infrequent under low-anthropogenic-impact conditions.

Although this study observed diverse yet low-abundance ARGs and VFGs, monitoring ecological security risks in Antarctica remains essential as human activities intensify in the region. Future work should integrate transcriptomics and proteomics to validate the expression and activity of ARGs and VFGs in extreme environments, thereby determining whether they pose a functional threat. These measures are crucial for preserving the Fildes Peninsula's scientific value and preventing further harmful impacts from human activities.

5 Conclusion

Using metagenome sequencing, our results demonstrated that the intertidal sediments of the Fildes Peninsula harbor a diverse but generally low-abundance resistome and virulome. Although a range of mobile genetic elements (ISs, integrases, viruses) carrying VFGs were detected, no ARGs were found on MGEs, and co-occurrence analyses indicate limited potential for horizontal dissemination. Additionally, the presence of opportunistic pathogens and multiple exotoxins/ exoenzymes, even at low abundance, highlights potential ecological and public health concerns that warrant targeted surveillance and management to prevent local amplification or spread. Given ongoing climate-driven mobilization and increasing Antarctic visitation, the detected baseline of ARGs/VFGs provides a crucial reference for future monitoring and designing measures to limit anthropogenic inputs and gene dissemination.

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Author contributions D. Y. analyzed data and wrote the manuscript with M. J. M. J. designed the study and reviewed the manuscript. L. L. collected the samples and designed the study. H.D. reviewed the manuscript.

Data availability Data are deposited in National Microbiology Data Center (NMDC, https://nmdc.cn/) with accession number NMDC10020391.

Competing interests The authors declare no competing interests.

References

Abbas A, Barkhouse A, Hackenberger D, et al. 2024. Antibiotic resistance: a key microbial survival mechanism that threatens public health. Cell Host Microbe, 32(6): 837-851, doi: 10.1016/j.chom.2024.05.015.

Adyari B, Liao X, Yan X H, et al. 2025. Anthropogenic gene dissemination in Tibetan Plateau rivers: sewage-driven spread, environmental selection, and microeukaryotic inter-trophic driving factors. Water Res, 284: 123887, doi: 10.1016/j.watres.2025.123887.

Andersson D I, Hughes D. 2014. Microbiological effects of sublethal levels of antibiotics. Nat Rev Microbiol, 12(7): 465-478, doi:10.1038/nrmicro3270.

Carattoli A, Zankari E, García-Fernández A, et al. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother, 58(7): 3895-3903, doi:10.1128/aac.02412-14.

Chen B W, Yuan K, Chen X, et al. 2016. Metagenomic analysis revealing Antibiotic Resistance Genes (ARGs) and their genetic compartments in the Tibetan environment. Environ Sci Technol, 50(13): 6670-6679, doi: 10.1021/acs.est.6b00619.

Chen S F, Zhou Y Q, Chen Y R, et al. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics, 34(17): i884-i890, doi:10.1093/bioinformatics/bty560.

Clark M S, Gregson B H, Greco C, et al. 2025. Assessing the impact of sewage and wastewater on antimicrobial resistance in nearshore Antarctic biofilms and sediments. Environ Microbiome, 20(1): 9, doi:10.1186/s40793-025-00671-z.

Deekshit V K, Srikumar S. 2022. 'To be, or not to be'-the dilemma of 'silent' antimicrobial resistance genes in bacteria. J Appl Microbiol, 133(5): 2902-2914, doi:10.1111/jam.15738.

Depta J, Niedźwiedzka-Rystwej P. 2023. The phenomenon of Antibiotic Resistance in the polar regions: an overview of the global problem. Infect Drug Resist, 16: 1979-1995, doi:10.2147/IDR.S369023.

Derakhshan S, Navidinia M, Haghi F. 2021. Antibiotic susceptibility of human-associated *Staphylococcus aureus* and its relation to *agr* typing, virulence genes, and biofilm formation. BMC Infect Dis, 21(1): 627, doi:10.1186/s12879-021-06307-0.

Dong R Y, Liu Y Q, Wang N L, et al. 2025. The distribution of antibiotic resistance and virulence factor genes in the sediment of Inexpressible Island, East Antarctica. Mar Life Sci Technol, 7(4): 978-988, doi:10.1007/s42995-025-00323-8.

Efimenko T A, Efremenkova O V, Demkina E V, et al. 2018. Bacteria isolated from Antarctic permafrost are efficient antibiotic producers. Microbiology, 87(5): 692-698, doi:10.1134/s0026261718050089.

El Ghachi M, Bouhss A, Blanot D, et al. 2004. The *bacA* gene of *Escherichia coli* encodes an undecaprenyl pyrophosphate phosphatase activity. J Biol Chem, 279(29): 30106-30113, doi:10.1074/jbc.M401701200.

Gabashvili E, Kobakhidze S, Chkhikvishvili T, et al. 2022. Metagenomic and recombination analyses of antimicrobial resistance genes from recreational waters of Black Sea coastal areas and other marine environments unveil extensive evidence for their both intrageneric and intergeneric transmission across genetically very diverse microbial communities. Mar Genom, 61: 100916, doi: 10.1016/j.margen.2021.100916.

Gou Y, Liu Y, Hu A Y, et al. 2025. Dissemination of genes associated with antibiotic resistance and bacterial virulence during ecosystem succession in two Tibetan glacier forefields. Sci Total Environ, 963: 178514, doi: 10.1016/j.scitotenv.2025.178514.

Guo J R, Bolduc B, Zayed A A, et al. 2021. VirSorter2: a multi-classifier, expert-guided approach to detect diverse DNA and RNA viruses. Microbiome, 9(1): 37, doi:10.1186/s40168-020-00990-y.

Gutiérrez J, González-Acuña D, Fuentes-Castillo D, et al. 2024. Antibiotic resistance in wildlife from Antarctic Peninsula. Sci Total Environ, 916: 170340, doi: 10.1016/j.scitotenv.2024.170340.

Hardie Boys M T, Pletzer D. 2025. A review of recently discovered mechanisms of cephalosporin resistance in *Pseudomonas aeruginosa*. Int J Antimicrob Agents, 66(2): 107527, doi: 10.1016/j.ijantimicag.2025.107527.

Hussain A, Mazumder R, Asadulghani M, et al. 2023. Combination of virulence and antibiotic resistance: a successful bacterial strategy to survive under hostile environments//Bacterial survival in the hostile environment. Amsterdam: Elsevier, 101-117, doi:10.1016/b978-0-323-91806-0.00004-7.

Hyatt D, Chen G L, Locascio P F, et al. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics, 11: 119, doi:10.1186/1471-2105-11-119.

Jara D, Bello-Toledo H, Domínguez M, et al. 2020. Antibiotic resistance in bacterial isolates from freshwater samples in Fildes Peninsula, King George Island, Antarctica. Sci Rep, 10(1): 3145, doi:10.1038/s41598-020-60035-0.

Jiang C X, Zhao Z L, Zhu D, et al. 2024. Rare resistome rather than core resistome exhibited higher diversity and risk along the Yangtze River. Water Res, 249: 120911, doi: 10.1016/j.watres.2023.120911.

Jiang X L, Ellabaan M M H, Charusanti P, et al. 2017. Dissemination of antibiotic resistance genes from antibiotic producers to pathogens. Nat Commun, 8: 15784, doi:10.1038/ncomms15784.

Joshi T, Xu D. 2007. Quantitative assessment of relationship between sequence similarity and function similarity. BMC Genomics, 8: 222, doi:10.1186/1471-2164-8-222.

Kang S J, Kim D H, Lee B J. 2024. Metallo-β-lactamase inhibitors: a continuing challenge for combating antibiotic resistance. Biophys Chem, 309: 107228, doi: 10.1016/j.bpc.2024.107228.

Ke Y C, Sun W J, Chen X L, et al. 2023a. Seasonality determines the variations of biofilm microbiome and antibiotic resistome in a pilot-scale chlorinated drinking water distribution system deciphered by metagenome assembly. Environ Sci Technol, 57(31): 11430-11441, doi: 10.1021/acs.est.3c01980.

Ke Y C, Sun W J, Jing Z B, et al. 2023b. Antibiotic resistome alteration along a full-scale drinking water supply system deciphered by metagenome assembly: regulated by seasonality, mobile gene elements and antibiotic resistant gene hosts. Sci Total Environ, 862: 160887, doi: 10.1016/j.scitotenv.2022.160887.

Kim H, Kim M, Kim S, et al. 2022. Characterization of antimicrobial resistance genes and virulence factor genes in an Arctic permafrost region revealed by metagenomics. Environ Pollut, 294: 118634, doi: 10.1016/j.envpol.2021.118634.

Li D H, Liu C M, Luo R B, et al. 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics, 31(10): 1674-1676, doi:10.1093/bioinformatics/btv033.

Li Z R, Li J, Cai W L, et al. 2019. Macrocyclic colibactin induces DNA double-strand breaks via copper-mediated oxidative cleavage. Nat Chem, 11(10): 880-889, doi:10.1038/s41557-019-0317-7.

Liu B, Zheng D D, Zhou S Y, et al. 2022. VFDB 2022: a general classification scheme for bacterial virulence factors. Nucleic Acids Res, 50(D1): D912-D917, doi:10.1093/nar/gkab1107.

Liu J W, Wan Q Q, Gaoxin D, et al. 2025. Hemolysin III drives neuroinvasion and meningitis while impacting desiccation resilience in *Cronobacter sakazakii*. Appl Environ Microbiol, 91(4): e00339-25, doi:10.1128/aem.00339-25.

Lou Z G, Xu H, Xia L J, et al. 2023. Enhanced freeze-thaw cycles facilitate the antibiotic resistance proliferation and dissemination risk under global climate change. Process Saf Environ Prot, 175: 119-128, doi: 10.1016/j.psep.2023.04.048.

Natarajan V P, Zhang X X, Morono Y, et al. 2016. A modified SDS-based DNA extraction method for high quality environmental DNA from seafloor environments. Front Microbiol, 7: 986, doi:10.3389/fmicb.2016.00986.

Nayfach S, Camargo A P, Schulz F, et al. 2021. CheckV assesses the quality and completeness of metagenome-assembled viral genomes. Nat Biotechnol, 39(5): 578-585, doi:10.1038/s41587-020-00774-7.

Nikaido H, Pagès J M. 2012. Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria. FEMS Microbiol Rev, 36(2): 340-363, doi:10.1111/j.1574-6976.2011.00290.x.

Niu C, Yu D, Wang Y L, et al. 2013. Common and pathogen-specific virulence factors are different in function and structure. Virulence, 4(6): 473-482, doi:10.4161/viru.25730.

Pärnänen K, Karkman A, Hultman J, et al. 2018. Maternal gut and breast milk microbiota affect infant gut antibiotic resistome and mobile genetic elements. Nat Commun, 9: 3891, doi:10.1038/s41467-018-06393-w.

Petrera A, Amstutz B, Gioia M, et al. 2012. Functional characterization of the *Mycobacterium tuberculosis* zinc metallopeptidase Zmp1 and identification of potential substrates. Biol Chem, 393(7): 631-640, doi:10.1515/hsz-2012-0106.

Qian X, Gunturu S, Guo J R, et al. 2021. Metagenomic analysis reveals the shared and distinct features of the soil resistome across tundra, temperate prairie, and tropical ecosystems. Microbiome, 9(1): 108, doi:10.1186/s40168-021-01047-4.

Santos A, Burgos F, Martinez-Urtaza J, et al. 2022. Metagenomic characterization of resistance genes in Deception Island and their association with mobile genetic elements. Microorganisms, 10(7): 1432, doi:10.3390/microorganisms10071432.

Segawa T, Takahashi A, Kokubun N, et al. 2024. Spread of antibiotic resistance genes to Antarctica by migratory birds. Sci Total Environ, 923: 171345, doi: 10.1016/j.scitotenv.2024.171345.

Setiabudy M, Widhidewi N W, Wijaya P A W, et al. 2024. Uncovering the enolase gene (*eno*) and its role in biofilm formation in clinical isolates of *Staphylococcus aureus*. Muhammadiyah Med J, 5(2): 97, doi:10.24853/mmj.5.2.97-106.

Siguier P. 2006. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res, 34(90001): D32-D36, doi:10.1093/nar/gkj014.

Tallada S, Hall G, Barich D, et al. 2022. Antibiotic resistance genes and taxa analysis from mat and planktonic microbiomes of Antarctic perennial ice-covered Lake Fryxell and Lake Bonney. Antarct Sci, 34(6): 408-422, doi:10.1017/s0954102022000360.

Thajudeen J, Venkatachalam S, Vipindas P V. 2025. Antibiotic resistome in the glacier forelands of polar regions. Appl Environ Microbiol, 91(6): e00762-25, doi:10.1128/aem.00762-25.

Tigabu A, Willcox M D P, Stapleton F. 2025. Phenotypic and genotypic profiling of antimicrobial resistance genes and virulence factors in *Pseudomonas aeruginosa* isolates from keratitis patients. Ocul Surf, 38: 392-420, doi: 10.1016/j.jtos.2025.10.011.

Van Goethem M W, Pierneef R, Bezuidt O K I, et al. 2018. A reservoir of ‘historical’ antibiotic resistance genes in remote pristine Antarctic soils. Microbiome, 6(1): 40, doi:10.1186/s40168-018-0424-5.

Wang F, Stedtfeld R D, Kim O S, et al. 2016. Influence of soil characteristics and proximity to Antarctic research stations on abundance of antibiotic resistance genes in soils. Environ Sci Technol, 50(23): 12621-12629, doi: 10.1021/acs.est.6b02863.

Wright G D. 2007. The antibiotic resistome: the nexus of chemical and genetic diversity. Nat Rev Microbiol, 5(3): 175-186, doi:10.1038/nrmicro1614.

Yin X L, Jiang X T, Chai B L, et al. 2018. ARGs-OAP v2.0 with an expanded SARG database and Hidden Markov Models for enhancement characterization and quantification of antibiotic resistance genes in environmental metagenomes. Bioinformatics, 34(13): 2263-2270, doi:10.1093/bioinformatics/bty053.

Yuan K, Yu K, Yang R Q, et al. 2019. Metagenomic characterization of antibiotic resistance genes in Antarctic soils. Ecotoxicol Environ Saf, 176: 300-308, doi: 10.1016/j.ecoenv.2019.03.099.

Zhang T, Ji Z Q, Li J, et al. 2022. Metagenomic insights into the antibiotic resistome in freshwater and seawater from an Antarctic ice-free area. Environ Pollut, 309: 119738, doi: 10.1016/j.envpol.2022.119738.

Zhao J, Duan G L, Zhu D, et al. 2024. Microbial-influenced pesticide removal co-occurs with antibiotic resistance gene variation in soil-earthworm-maize system. Environ Pollut, 342: 123010, doi: 10.1016/j.envpol.2023.123010.

Zhou Z M, Zhu R B, Song Y X, et al. 2024. Penguin-driven dissemination and high enrichment of antibiotic resistance genes in lake sediments across Antarctica. Environ Sci Technol, 58(32): 14460-14474, doi: 10.1021/acs.est.4c02732.

Zhu D D, Fan Y F, Wang X Y, et al. 2023. Characterization of molecular chaperone GroEL as a potential virulence factor in *Cronobacter sakazakii*. Foods, 12(18): 3404, doi:10.3390/foods12183404.

Zhu Y G, Zhao Y, Li B, et al. 2017. Continental-scale pollution of estuaries with antibiotic resistance genes. Nat Microbiol, 2: 16270, doi:10.1038/nmicrobiol.2016.270.