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Biological Sciences

Identification of mobile resistome in soils exposed to different impacts in Fildes Peninsula, King George Island

Matías Giménez[1,2,3], Gastón Azziz[1,4] and Silvia Batista[1]

[1]Molecular Microbiology Laboratory, BIOGEM, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay; [2]Microbial Genomics Laboratory, Institut Pasteur Montevideo, Montevideo, Uruguay; [3]Center for Innovation in Epidemiological Surveillance, Institut Pasteur Montevideo, Montevideo, Uruguay and [4]Microbiology Laboratory, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay

Abstract

Antimicrobial resistance is one of the most important global health issues identified in recent decades. Different approaches have been used to establish the presence and abundance of antimicrobial resistance genes (ARGEs) in the environment. In this study, we analysed soil samples from Fildes Peninsula (King George Island, Maritime Antarctica) exposed to human and bird impacts. The objective of the work was to identify ARGEs in the samples and to evaluate whether these genes were located in plasmids using two different strategies. A metagenomic analysis was employed to identify ARGEs according to the CARD database and to determine whether they were associated with plasmidic sequences. The analysis showed that the site exposed to high anthropogenic activity had a higher number of ARGEs compared to other sites. A large percentage of those ARGEs (19.4%) was located in plasmidic contigs. We also assessed replicon mobilization using microbial communities from these soil samples as donors through an exogenous plasmid isolation method. In this case, we could recover plasmids with ARGEs in a Tc^r transconjugant clone. Although they could not be fully assembled, we could detect broad host range IncP1 and IncQ plasmid sequences. Our results indicate that sewage-impacted soils could be hotspots for the spread of ARGEs into the Antarctic environment.

Key words: antimicrobial resistance genes, metagenomics, plasmids, resistome

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Introduction

Antimicrobial resistance (AMR) occurs when a bacterium is not affected by a specific compound to which other microorganisms are susceptible. This evolutionary phenomenon is exacerbated by the misuse of antimicrobials, significantly impacting public health over the last few decades (O'Neill 2016). Antimicrobial resistance genes (ARGEs), usually identified in clinical settings, farms, sewage water and urban environments, arise as a consequence of a strong selective pressure associated with the use of antibiotics in human health and animal production. There is a consensus on the approach to tackle this public health issue that considers the ability of many bacteria to thrive in different environments. The One Health concept takes into account the interconnection of animal, environmental and human microbiomes to understand this complex adaptive bacterial process (Aslam *et al.* 2021).

The Antarctic environment can be a source of novel ARGEs (Azziz *et al.* 2019). Most of these naturally occurring genes have evolved for other metabolic purposes, but as a result of substrate similarity they can metabolize antimicrobial compounds with low efficiency (Martínez *et al.* 2007). ARGEs existed prior to the use of antimicrobials, as well as to the identification of resistance in clinical settings. This has been evidenced by the detection of clinical-type ARGEs in ancient DNA purified from isolated permafrost samples (D´Costa *et al.* 2011). Clinical-type ARGEs are highly effective at the metabolization of antimicrobial compounds and are frequently found to be associated with clinical settings. In addition, a broad diversity of ARGEs has been detected in natural, low-impacted environments, suggesting that different compounds with potential antimicrobial activity could play a role in those communities (Scott *et al.* 2020). Several human pathogens can survive in natural environments not associated with their hosts (Struve & Krogfelt 2004, van Elsas *et al.* 2011, Crone *et al.* 2020). For example, sewage systems are settings where human-associated bacteria come in contact with environmental microorganisms. These sites have been described as hotspots for ARGE release and dispersion into the environment (Delgado-Blas *et al.* 2021, Vasallo *et al.* 2021).

Plasmids are genetic elements that can regulate their replication independent of the host chromosome. Additionally, plasmids may contain genes that confer the ability to conjugate with other organisms. They can also integrate other intra-genomic mobile sequences, such as insertion sequences, transposons and integrons, which enhance their ability to capture and spread genes conferring adaptive advantages, such as antibiotic resistance genes (ARGs) or metal resistance genes (MRGs; Di Cesare *et al.* 2016, Che *et al.* 2021). Plasmids are recognized as common carriers of ARGEs in clinical settings (Rozwandowicz *et al.* 2018). Consequently, genomic surveillance of plasmids carrying ARGEs is becoming essential to understand and monitor pathogen outbreaks in clinical settings.

Corresponding author: Matías Giménez; email: mgimenez@pasteur.edu.uy

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The Antarctic continent has various regions with characteristic climates and associated biota. Classically, three biogeographical regions have been identified, which include the very cold and dry Continental region, the Maritime Antarctic region, with a less harsh climate, and the sub-Antarctic region, with milder weather and a characteristic biota (Convey 2011). Antarctica is subject to strict regulations in environmental management declared in the Environmental Protocol to the Antarctic Treaty (https://www.ats.aq/e/protocol.html). Thus, human activities that take place on the continent must be associated with an environmental impact assessment, defining restricted access sites, identified as Antarctic Specially Protected Areas (ASPA). These sites have been selected considering various aspects, such as the presence of fossils, animal nests and history, amongst others.

The South Shetland Islands are located within the biogeographical region identified as Maritime Antarctica. King George Island stands out for hosting the highest number of scientific stations per area compared with the rest of the continent. Therefore, this island is considered one of the most densely populated sites of the Antarctic continent, at least during the summer, and there are reports of AMR presence associated with the human impact in this region (Hwengwere *et al.* 2022). However, this island hosts nests and colonies of numerous marine mammals and birds. The fauna of this island, especially birds, have also been linked to the spread of AMR bacteria, given their migration patterns and eventual contact with human populations (Cerdà-Cuéllar *et al.* 2019).

Fildes Peninsula, the largest area of King George Island without permanent ice cover, represents a small area of the Antarctic continent containing differentially impacted sites. Taking this into consideration, we aimed to evaluate the admixture of bacteria and their genetic determinants of resistance in soil microbial communities exposed to bird and human impacts. Specifically, the objective of this work is to describe the clinical resistome of samples obtained from these sites using a metagenomic approach and to assess the potential transfer of ARGEs in these microbial communities. The analysis of metagenomic data is complemented by the identification of MRGs and their association with mobile genetic elements.

Materials and methods

Sample collection and metagenomic sequencing

Nine soil cores were collected in Fildes Peninsula during the summer campaign of 2017. Three sites were selected with the intent to collect soil exposed to different environmental conditions, especially considering influences of biotic origin (Fig. S1). The first site, named IA, is located in Ardley Island (62°12′34″S, 58°55′44″W). Some years ago, intending to protect the birds inhabiting this area, this island was classified as ASPA 150. This site hosts a large bird colony that has been monitored for a long time, and the soil is considered to be intensively impacted by bird activity (ornithogenic soil; Guo *et al.* 2018). The second sampled site, named HTP (ASPA 125d), is located at Half Three Point (62°13′38″S, 58°57′12″W). We selected this site as it is relatively far from scientific research stations and breeding sites for mammals and birds, assuming that it was less exposed to animal impact. Finally, samples next to Artigas Research Scientific Station (BCAA) were also collected, specifically from soils impacted by the wastewater treatment system, which has previously been described as not completely isolated from the Antarctic environment (Tort *et al.* 2017). These samples were designated BCAA (62°11′04″S, 58°57′54″W).

Three soil cores, separated by a distance of at least 2 m from each other, were aseptically collected at each sample site and kept at 4°C until further processing at the laboratory in Uruguay. The total DNA community was extracted using a PowerSoil DNA kit from Qiagen (Hilden, Germany) following the manufacturer's instructions. Extracted DNA quantity and integrity were assessed by 0.9% (w/v) agarose gel electrophoresis in tris acetate EDTA (TAE) buffer. The extraction that presented the best quality profile (both in quantity and integrity) from each of the three replicas was used for metagenomic sequencing at Macrogen, Inc. (Seoul, South Korea). Illumina technology was used through a HiSeq2500 sequencer, which yielded more than 2 Gbp of data for each metagenome with a read length of 101 bp. Raw reads generated from these samples were deposited in at the National Center for Biotechnology Information (NCBI) in the Bioproject PRJNA1046061.

Metagenomic analysis

Metagenomic read quality was assessed using *FastQC* software (Andrews 2010). Reads were filtered and trimmed using *Trimmomatic* software (LEADING:3 TRAILING:3 SLIDINGWINDOW: 4:15 MINLEN:80; Bolger *et al.* 2014). The remaining and corrected reads were used as input for metagenomic assembly using *IDBA-UD* software with default parameters. *Quast* software (Gurevich *et al.* 2013) was used to assess the quality of the assemblies. Plasmid-derived sequences were detected using the *plaSquid* pipeline (Giménez *et al.* 2022; https://github.com/mgimenez720/plaSquid). Plasmid replication initiator proteins were retrieved using the *-ripextract* option and compared against the NCBI non-redundant protein database. *Abricate* software, using the CARD database with 70% identity and coverage thresholds, was used to detect ARGEs in plasmidic and metagenomic contigs (https://github.com/tseemann/abricate).

To understand the dynamics of resistance *via* a genome-centred approach, we also ran *MetaWRAP* software (Uritskiy *et al.* 2018). This wrapper software groups metagenomic contigs in bins composed of sequences derived from the same species genomes, called metagenome-assembled genomes (MAGs). *CheckM* software was also run to filter out the assembled MAGs by completeness (< 50%) and contamination (> 10%; Parks *et al.* 2015). MRGs were searched by using the experimentally confirmed resistance genes of the BacMet database (v. 2.0; Pal *et al.* 2014), the blastp algorithm from *BLAST+* software (v. 2.12.0) and filtered to 75% of identity and coverage using custom scripts (Altschul *et al.* 1990).

To assess human impact in the sequenced microbial communities, the crAssphage genome was used as an indicator of human impact. The crAssphage genome was used as a reference for mapping with *bowtie2* using default parameters (Langmead & Salzberg 2012).

Construction of a strain for exogenous plasmid isolation

A recombinant strain derived from *Escherichia coli* DH5α expressing kanamycin resistance (Km^r) and GFP protein (*gfp* gene) was constructed. For this, a mini-transposon mTn5-*gusA-nptII-pgfp12* (Xi *et al.* 1999) was used to insert the aforementioned genes into the chromosome of *E. coli* DH5α. This was done by biparental conjugation using *E. coli* S17.1-λpir with the plasmid pUT::mTn5-*gusA-nptII-pgfp12* as a donor. This plasmid can only replicate in λpir strains able to express π protein. Resistant

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(Nal^r, Km^r) transconjugants were selected, intending to work with recombinant DH5α clones in which just the mini-transposon mTn5-*gusA-nptII-pgfp12* was inserted into the chromosome. Three clones were selected and further replicated in Luria-Bertani (LB) solid medium, and the stability of the fluorescent phenotype was checked.

Exogenous isolation assay

The recombinant fluorescent strain derived from *E. coli* DH5α carrying the *gfp* gene in the chromosome was used as an acceptor for an *in vitro* exogenous isolation assays. We grew 5 mL of cell cultures for 24 h in LB broth at 37°C and stirred them at 200 rpm. The entire culture was centrifuged at 3900 *g* for 5 min and resuspended in 1 ml LB broth. The microbial communities contained in nine soil samples were used as conjugation donors, and three samples obtained from each site were evaluated. For this, 1 g of soil was resuspended in 9 ml of 1/10 diluted sterile tryptic soy broth (TSB) with five autoclaved glass spheres of 5 mm diameter and stirred for 90 min at 200 rpm and 37°C to disaggregate soil particles. A volume of 4 ml of soil suspension was thoroughly mixed with 1 ml of resuspended recipient cells and centrifuged at 3900 *g* for 5 min. The mixture was resuspended in 100 µl of LB. These conjugation mixtures were thoroughly pipetted over a 45 µm pore-size filter, placed on LB agar and incubated for 48 h at 25°C (Kopmann *et al.* 2013). This procedure was repeated for all nine soil samples, with three samples from each site used as donor communities.

Then, filters were washed with 5 ml of 0.85% (w/v) NaCl. Three serial dilutions were prepared from this suspension, and volumes of 100 µl were plated on LB agar supplemented with kanamycin (50 µg/ml; Km), nalidixic acid (25 µg/ml; Nal), cycloheximide (10 µg/ml; Chm) and one of the following antibiotics: tetracycline (10 µg/ml), ampicillin (50 µg/ml), trimethoprim (25 µg/ml) or gentamycin (10 µg/ml). Plates were incubated at 37°C for 24 h and fluorescent colonies were identified and selected under ultraviolet light.

Genomic sequencing analysis of the receptor strain

Fluorescent colonies grown on LB agar Nal Km Chm plus one of the previously mentioned antibiotics were transferred to fresh media to confirm their phenotypic stability. Selected colonies were grown in 5 ml LB broth with the corresponding antibiotics. Genomic DNA was extracted using a Quick-DNA Fungal/Bacterial Miniprep kit (Zymo Research, USA) following the manufacturer's instructions, and DNA integrity and quantity were checked *via* 0.95% agarose gel electrophoresis. Genomic DNA was sequenced at the facilities of Genoma Mayor, Universidad Mayor (Santiago, Chile) using Illumina technology. Raw reads were trimmed using the same software and parameters as previously described for metagenomic data. Filtered reads were mapped against the *E. coli* DH5α (GCA_000755445.1) reference assembly and miniTn5 assembly (HQ328084.1) and filtered out. The remaining reads were assembled using *SPAdes* software (Bankevich *et al.* 2012). Additionally, *plaSquid* software was used to detect and classify plasmidic contigs, which were further analysed by looking for ARGEs and MRGs as previously described.

To detect plasmidic contigs retrieved through exogenous isolation in the metagenome sequenced from the donor sample, metagenomic reads were mapped against plasmidic contigs using *BWA* software (Li & Durbin 2010). Additionally, the *SAMtools* toolkit was used to compute the coverage of plasmidic contigs with quality-filtered metagenomic reads (Li *et al.* 2009).

Results

Genome-centric resistome analysis

We assembled a different number of contigs for each sequenced metagenome. N50 values ranged from 1280 bp in the HTP metagenome to 3506 bp in the BCAA metagenome. The total length of assembled metagenomes also varied between 33 and 103 Mbp. The presence of human faecal contamination was analysed by mapping these metagenomes against the crAssphage genome as an indicator of human impact (data not shown). This analysis showed that the BCAA sample was the only one containing reads that could be assigned to this phage. Additionally, through taxonomic profiling of the communities, we detected the presence of *Faecalibacterium*, *Prevotella*, *Acinetobacter* and other genera associated with the human gut microbiome in BCAA (Fig. S2).

In order to analyse the genomic resistance traits of the most abundant genomes in the samples, we assembled MAGs from shotgun metagenomic data (Fig. 1). We could identify a total of 20 medium- and high-quality MAGs; 19 of them (95%) included MRGs, while seven encoded ARGEs (35%). Most of the MAGs detected were classified at the family or genus level, corresponding to environmental bacteria. The BCAA metagenome had more MAGs identified. Five of them were classified within the Comamonadaceae family and another two were within the *Flavobacterium* genus (Fig. 1). Additionally, we detected two genomes of Pseudomonadaceae and *Pseudomonas* in the BCAA sample. The *Psychrobacter* genus was also present in two of the three metagenomes analysed. Additionally, an Aeromonadaceae MAG, found in the IA sample, had the most resistance traits identified, with 30 different resistance genes, mainly MRGs. The only MAG detected without known resistance traits corresponded to *Helicobacter* genus and was found in the IA sample.

Gene-centric resistome analysis

Figure 2 shows all ARGEs (38 genes encoding resistance against nine different antibiotic classes) identified in the metagenomes of the three samples analysed. BCAA contains a higher number of diverse ARGEs compared to the other two samples. This was the only metagenome in which we could detect ARGEs encoded in plasmidic contigs. In fact, nearly 20% of ARGEs found, encoding resistance to seven different antibiotic types, were assigned to plasmidic contigs. Within this metagenome, genes for resistance to trimethoprim and tetracycline were found exclusively in plasmidic contigs. Macrolide resistance genes were also evenly distributed in chromosomal and plasmidic contigs.

Diverse kinds of ARGEs were found in the three metagenomes, encoding resistance to different antibiotics. Most of them are components of non-specific resistance mechanisms, which could confer resistance to multiple drugs. Various aminoglycoside resistance genes were found, some of them evenly distributed across samples, such as the *aadA* gene. However, the gene *aph*(3′)-*IIa* was found in metagenomes IA and HTP but not in BCAA. In turn, in BCAA we could find a gene encoding a variant of the enzyme APH(3′)-Ia in a plasmidic contig highly similar to the *Acinetobacter baumanii* pAC30b plasmid (CP007579.1). Beta-lactam resistance was also an important trait found in these Antarctic metagenomes. TEM-4 was only found in metagenome IA, while OXA and VEB-1 variants were found in

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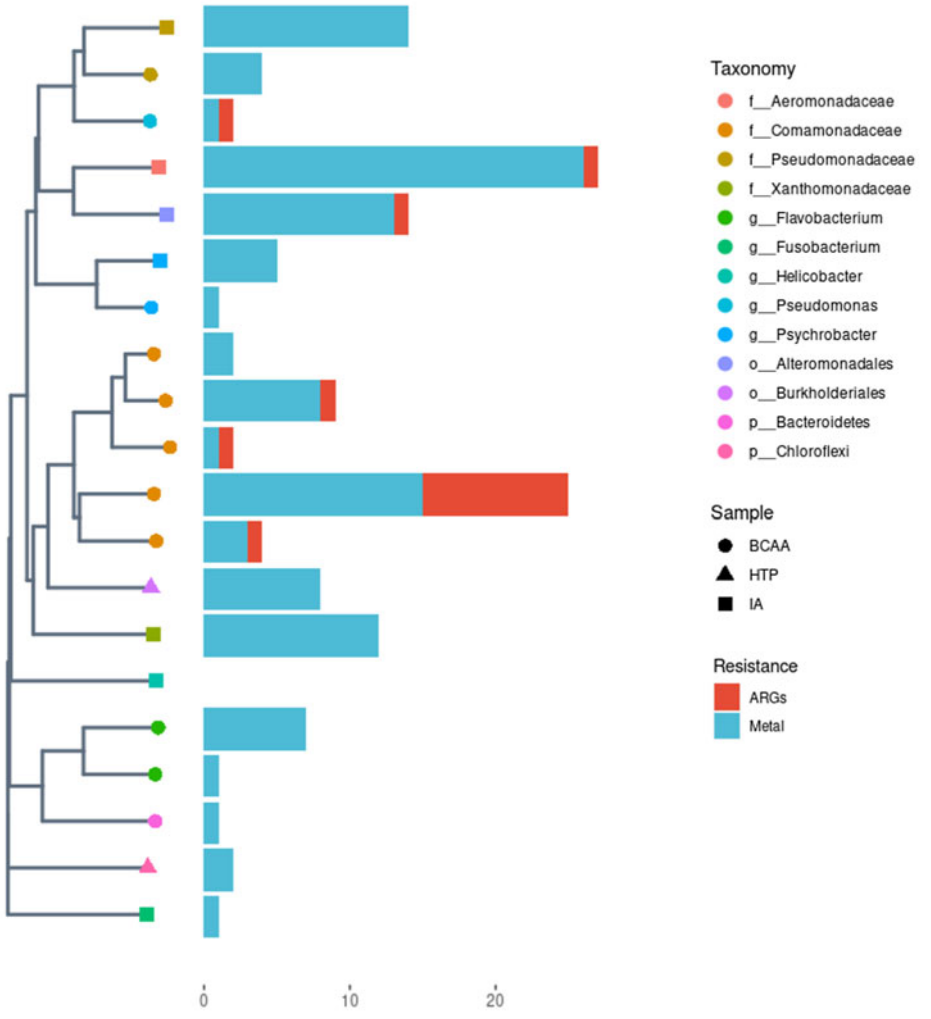

Figure 1. Phylogenetic tree of metagenome-assembled genomes (MAGs) detected by *MetaWRAP* software using 43 marker genes detected with *CheckM* software. Concatenated genes at the protein level were used for phylogenetic tree construction with the Neighbour-Joining algorithm in the *ape R* package. Tip colours represent the taxonomic classification assigned by *MetaWRAP* software and tip shapes indicate the sample from which the MAG was assembled. Metal and antibiotic resistance genes (ARGs) for each MAG are represented as light blue and red bars, respectively. BCAA = Artigas Research Scientific Station.

BCAA. The *OXA-15* gene was found in a highly fragmented plasmidic contig. Macrolide resistance was another trait found in the BCAA metagenome. Genes *mphD* and *msrE* were located in the same plasmidic contig, with high sequence identity to a region of megaplasmid pXBB1-9 hosted in *Acinetobacter johnsonii* XB1. Additionally, sulphonamide resistance genes were detected

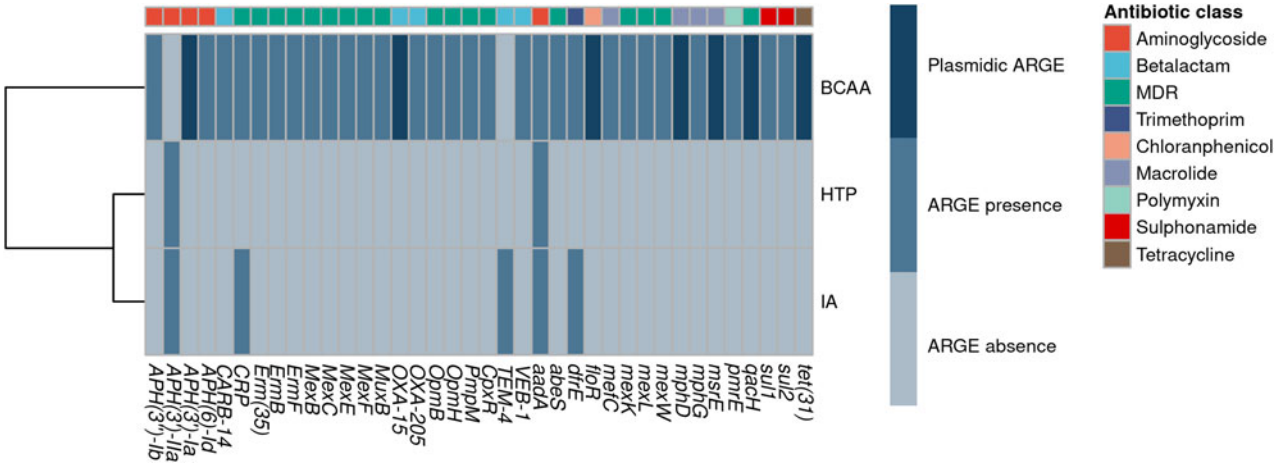

Figure 2. Tile plot of antimicrobial resistance genes (ARGEs) in the three metagenomes analysed. ARGEs encoded in plasmidic contigs, detected by *plaSquid* analysis, are indicated in navy blue. Colours in the top bar indicate the antibiotic class for each detected ARGE. BCAA = Artigas Research Scientific Station; MDR = multi-drug resistance.

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in chromosomal contigs, and a tetracycline resistance gene was detected in a plasmidic contig, both in the BCAA metagenome.

In order to compare the presence of resistance genes between samples, we normalized the count of ARGEs and MRGs relative to the 16S rRNA count. This analysis demonstrated the significant presence of MRGs in the metagenomes of the three samples analysed, with a mean value of 1.5 genes per 16S rRNA gene (Fig. S3). When considering only plasmidic contigs, the normalized MRG count were decreased by more than 17-fold for the BCAA metagenome. The main traits found confer resistance to multiple metals such as copper, zinc, cadmium, arsenic and lead. In the case of plasmidic ARGEs, these were only detected in BCAA-derived plasmid sequences (Fig. 2).

Exogenous plasmid isolation assay

The samples collected were used for an *in vitro* exogenous plasmid isolation assay to determine whether the ARGEs contained in these soil samples could be potentially transferred to human-associated bacteria. Nine soil microbial communities were used as donors, and *E. coli* DH5α::mTn5-*gusA-nptII-pgfp12* was used as a receptor strain. We could detect the acquisition of resistance to tetracycline antibiotics in one of the nine transconjugation assays; three colonies were seen to grow with a fluorescent phenotype and resistance to tetracycline (Fig. S4). In this case, one transconjugant strain was sequenced along with cargo plasmids. In order to facilitate plasmid detection and assembly, reads mapping against the *E. coli* chromosome were discarded. Plasmidic contigs were detected with *plaSquid* software, and their classifications and traits are shown in Table I. Multiple plasmidic replication origins were found in the transconjugant strain. Three of them could be classified in replicon types IncP1, IncQ and ColE1. These three replicons have different mobility characteristics. MOBP1 and MOBQ relaxases were found in different contigs, which demonstrates the mobilization capacity of some of these replicons.

The largest contig encodes an IncP1 replicon that is part of a self-mobilizable plasmid, given the presence of the type IV secretion system (TIVSS) genes (Fig. 3a). This was the only self-conjugative replicon found in the transconjugant strain. However, we could not find any ARGE in this 43 kb contig. Notably, we could find a tetracycline resistance gene *tetC* in a 1345 bp contig (Contig-31; Table I). This was the only selected resistance phenotype included in the transconjugation assay, and it could not be linked to any particular replicon.

Another interesting contig identified was an IncQ-like plasmid, whose entire replicon could be assembled and had a coverage of more than 7X in the metagenome of BCAA site (Contig-4; Table I). This mobilizable plasmid encodes for a relaxase but lacks a TIVSS. The only TIVSS detected was the one associated with the IncP1 replicon. In addition, two aminoglycoside resistance genes could be found in this replicon. One of them, *rmtG*, encodes a ribosomal methylase that gives high levels of aminoglycoside resistance (Fig. 3b). This phenotype was verified by the growth of the transconjugant strain in LB medium supplemented with 250 μg/ml of kanamycin compared to the lack of growth of the receptor strain, which includes the *nptll* gene. Another aminoglycoside resistance gene, *aadA6*, and a multidrug efflux pump, *qacH*, were detected in a 2187 bp contig (Contig-12; Table I). Plasmidic ARGEs were not located in the same contigs as the replicon sequence determinants, given the high fragmentation of plasmidic sequences.

Discussion

The Antarctic continent features a variety of regions with distinct climates and associated biota. Traditionally, some islands surrounding the continent and the West coast of the Antarctic Peninsula have been grouped into a biogeographical region known as Maritime Antarctica. Unlike the colder and drier Continental region, Maritime Antarctica has a milder climate, which supports a unique biota that has been increasingly impacted by global climate change (Convey 2011) and local activities from scientific stations in the area. According to previous studies (Tin *et al.* 2009), the presence of numerous research stations in certain sites is associated with a significant environmental impact. Global warming also affects microbial communities in Antarctic soils, leading to observable trends. All of these factors influence the evolution and composition of microbial communities in this region.

Sewage treatment and disposal policies in the Antarctic continent are under discussion today. It has been demonstrated that non-treated sewage deposition into sea water may have a significant impact on marine wildlife (Stark *et al.* 2015). Additionally, there have been previous reports of human microbiome-associated bacteria being delivered into the environment from the sewage waters of several Antarctic research stations (Power *et al.* 2016, Hernández *et al.* 2019). In this study, we found a greater number of various clinical-type ARGEs in the BCAA sample compared to at the

Table I. Plasmidic contigs sequenced from transconjugant strain *Escherichia coli* DH5αGfp^{+}.

Contig	RIP domain	MOB group	Rep type	Length (bp)	Metagenomic coverage (X)	Resistance genes
Contig-1	TrfA	MOBP1	IncP	43 383	2.3752	ND
Contig-15	Rop	ND	ND	1909	0	ND
Contig-4	RepC	MOBQ	IncQ	7527	7.5709	*rmtB*, *rsmI*
Contig-13	ND	ND	ColRNDI	2121	3.7807	ND
Contig-3	ND	MOBP1	ND	11 349	1.8938	ND
Contig-12	ND	ND	ND	2187	0	*qacH*, *aadA6*
Contig-11	ND	ND	ND	2210	1.85	*dfrB1*, *cmlA1*
Contig-31	ND	ND	ND	1345	1.27	*tetC*
Contig-6	ND	ND	ND	4703	4.64	*sul1*

MOB = mobilization; ND = not detected; Rep = replicon; RIP = replication initiator protein.

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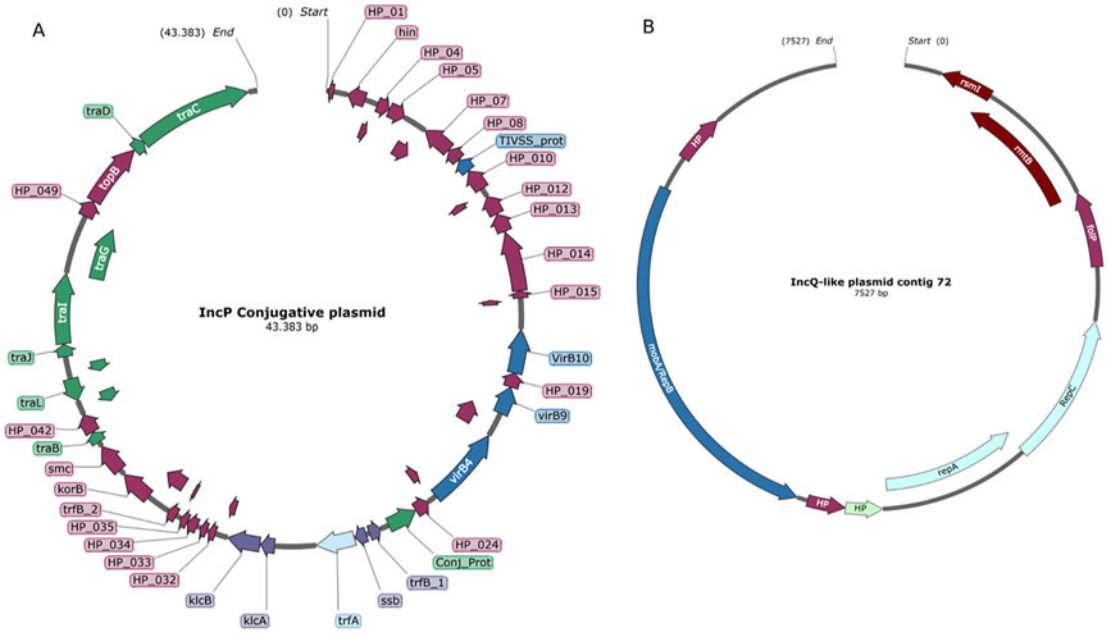

Figure 3. Diagram of annotated plasmids that could be assembled from sequencing of transconjugant clones obtained by exogenous plasmid isolation assays. The IncP plasmid contains the modules needed for self-conjugation, while the IncQ-like plasmid contains a relaxase gene that makes it mobilizable.

other two sites. This sample was collected from soil near a septic chamber, where there had been previously reported leaking events during high-occupancy periods, which directly dispersed human microbiome components into the environment (Tort *et al.* 2017). Additionally, samples were taken during the summer when scientific stations have high occupancies of scientific and maintenance staff. Environmental monitoring protocols should be established to quantify this kind of impact. In addition, the installation of a wastewater treatment plant is being evaluated as a future operation at BCAA.

Some of the MAGs recovered belong to taxonomic groups that have been reported and studied in the Antarctic environment. For instance, *Psychrobacter* and *Flavobacterium* are commonly found in the Antarctic environment. Several bacteria belonging to these genera are adapted to extreme conditions, and on occasions some isolates were studied for biotechnological applications (Herrera *et al.* 2019, Acevedo-Barrios *et al.* 2022, Paun *et al.* 2022). The impact of penguins on Ardley Island soil samples could be confirmed by the presence of a *Fusobacterium* MAG in the assembled metagenome. This genus has been reported as dominant in the gut microbiome of Adélie and gentoo penguins established on this island (Zeng *et al.* 2021). In addition, metal resistance traits seem to be widely distributed in bacteria that are dominant in the three samples analysed. For example, we could detect several traits of metal resistance in environmental bacteria such as *Burkholderia*. Interestingly, the highest load of MRGs was detected in a MAG belonging to the Aeromonadaceae family, present in the sample collected from the ornithogenic soil of Ardley Island. Penguin colonies have been demonstrated to accumulate high concentrations of metals in faeces and feathers; thus, MRGs could be an adaptive trait for thriving in ornithogenic soils (Romaniuk *et al.* 2018, Castro *et al.* 2021).

This study represents the first effort to assess the mobilization capacity of clinical ARGEs detected in Antarctic soils exposed to various types of environmental impact. We identified 36 classes of ARGEs in a soil sample collected near the septic chamber of BCAA, a higher number compared to the ARGEs identified in the other two metagenomes. Additionally, we determined that some of these resistance genes are associated with mobile genetic elements and are potentially transferable to exogenous bacterial acceptors.

We obtained a transconjugant clone of *E. coli* using the microbial community contained in a soil sample from BCAA as a donor. Although optimal conditions of temperature and incubation time were not adjusted to the Antarctic environmental conditions, we found that some plasmids could be mobilized to another recipient bacteria. In these community-wide conjugation experiments, we mobilized together different types of plasmids, ranging from totally self-conjugative, such as the IncP1 replicon, to other plasmids containing just an OriT as a mobilization element, such as the ColEI replicon. These multiple-replicon transfer events catalysed by the presence of IncP1 conjugative plasmids have already been reported in other environments (Schlüter *et al.* 2007, Brown *et al.* 2013). Even more interesting is the fact that IncP1 backbones with high similarity to the pKJK5 plasmid, such as the one reported in this work, seem to be highly effective at thriving in soil microbiomes and at mobilizing ARGEs (Heuer *et al.* 2012).

Another remarkable feature of IncP1- and IncQ-like replicons is their broad host range, which enables ARGE transfer between phylogenetically distant bacterial species. This may be a key step for ARGE dispersal among different environmental compartments (Smalla *et al.* 2000, Klümper *et al.* 2015). Soil microbial communities impacted by sewage or manure are important hotspots for ARGE transfer events, given the ecological connectivity of microbial communities that have evolved and adapted to the different environments (Cohen *et al.* 2011, Martínez *et al.* 2015). Additionally, there is evidence for the presence and release of antibiotics in Antarctic research station sewage, which could further act as a selective pressure for newly resistant clones

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(Hernández *et al.* 2019). Policy frameworks should take into consideration the microbiological risk for the selection of ARGEs within the Antarctic microbiosphere (Hernando-Amado *et al.* 2019).

Our results suggest that the high diversity of clinical-type ARGEs and the presence of human gut bacteria indicate significant microbiological impacts from inadequate sewage infrastructure at Antarctic research stations. Additionally, anthropogenic impacts on soil microbiomes include the potential transfer of mobile genetic elements, which could drive the evolution of indigenous microbial communities and threaten the genetic diversity of Antarctic soil microbiomes. Future research should include a quantitative evaluation of ARGEs and specific bacterial genes as indicators, using soil and water samples collected at predefined sites and throughout the year (Berendonk *et al.* 2015). With the planned installation of a new effluent treatment system at BCAA, such analyses could be integrated into routine monitoring procedures.

Author contributions. MG conceived the project, collected the samples, conducted the analyses and wrote the first draft of the manuscript. GA contributed to the analysis of the molecular dataset and to the editing of the final manuscript. SB conceived the project, obtained funding resources and contributed to the editing of the manuscript.

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Competing interests. The authors declare none.

Supplemental material. Four supplemental figures will be found at https://doi.org/10.1017/S0954102024000300.

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