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*Article*

# Antimicrobial Resistance Elements in Coastal Water Identified from Llanquihue Lake, Chile

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**Abstract:** Antimicrobial resistance has been stated to be a global health problem. In Chile, the use of antibiotics should be declared by medical prescription, however, it is unknown what happens to the drugs once the treatment ends. One possibility is that these end up being disposed of in the trash or down the drain. In both scenarios, antibiotics could accumulate in the environment, stimulating the emergence of antimicrobial resistance mechanisms and their transfer between microorganisms. Unfortunately, sometimes wastewater ends up in bodies of water, due to the dragging of elements by rain, or by the presence of illegal water discharges. In this work, we use shotgun metagenomics to elucidate the functional and microbial composition of biohazard elements in the bay of Puerto Varas City, Chile. As expected, we found a high diversity in microbial communities, with bacterial elements described as human or animal pathogens. Also, we detect a diverse repertory of virulence and antibiotic-resistant genes (ARGs), related with macrolides, beta-lactams, and tetracyclines resistance, which are consistent with the families of antibiotics most used in Chile. Similar ARGs were identified in DNA mobile elements. In addition, we tested the AMR ability in 20 bacterial strains recovered from the Llanquihue lake. This is the first report of the presence of genomic elements that could constitute a health problem for the people who live around the Llanquihue Lake of Chile, considering the importance of the interconnection between environmental, animal, and human health, a concept known as One Health.

**Keywords:** Antimicrobial resistance; metagenomics; DNA mobile elements

## 1. Introduction

The emergence and spread of antimicrobial resistance (AMR) is a major global health problem [1]. The World Health Organization (WHO) has classified it as a major threat to global public health [2]. AMR occurs when microbes, such as bacteria, fungi, viruses, and parasites, become resistant to the drugs used to treat them [3]. This makes it difficult or even impossible to treat infections caused by antimicrobial resistant microorganisms [4].

The increment in antimicrobial resistant mechanism and transmission of resistance genes from environmental bacteria to pathogenic strains is an imperative threatening to human and animal health which deserves the public and governmental attention [5]. AMR has economic consequences, its impacts include increased healthcare costs, lost productivity and overall economic burden [6].

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Antimicrobial resistance can lead to increased costs of treating resistant bacterial infections. The economic burden goes beyond healthcare costs and includes reduced income due to prolonged illness and premature death, affecting both individuals and society [7]. In low- and middle-income countries, where infectious diseases are most prevalent, the failure of first-line antibiotics has led to increased mortality and costs. In addition, the economic impact of antimicrobial resistance affects not only humans, but also animals, leading to economic losses in animal husbandry and further straining economies [8].

There are many factors that contribute to the development and spread of AMR, including the overuse and misuse of antibiotics in human and animal clinical pharmacotherapy, the indiscriminate use of antibiotics in animal production as growth promoters, poor sanitation and hygiene of health care systems, and the improper disposal of waste contaminated with antibiotics and resistant bacteria [9]. One of the leading contributors to the dissemination of AMR is the presence of antimicrobial resistance genes (ARGs) in environmental reservoirs, such as lakes, rivers, and oceans [10]. Also, Mobile Genetic Elements (MGE) play a significant role in the transference and dispersion of Antimicrobial Resistance Genes (ARGs) among bacteria. They facilitate horizontal gene transfer (HGT) by various mechanisms, such as virions, conjugative systems, and plasmids. MGEs can carry ARGs and transfer them to other bacteria, contributing to the spread of antibiotic resistance [11]. Understanding the prevalence and distribution of ARGs and MGE in these aquatic ecosystems is crucial for developing strategies to mitigate their potential impact on public health. The resistome consists of all ARGs, including those circulating in both pathogenic and non-pathogenic bacteria [12], and aquatic environments has been already reported as reservoir of these ARGs elements [13,14].

Llanquihue Lake is the second largest lake in Chile, with a surface area of approximately 860 km2 and a maximum register depth of 317 m. The lake is situated in the Llanquihue Basin, which is bounded by the Andes Mountains to the east and the Coastal Range to the west. It is surrounded by several cities and towns, each with its own unique charm and attractions [15]. Puerto Varas, located on the eastern shore of the lake, is a popular tourist destination, with an urban population of around 26.000 inhabitants, based on the 2017 census of Chile. Given the significant anthropogenic activities and the growing concern regarding AMR, it becomes imperative to investigate the presence and characteristics of ARGs and MGE in the coastal waters of Llanquihue Lake. By investigating the presence of ARGs elements in coastal water recovered from Puerto Varas shore, this study aims to shed light on the prevalence, distribution, and potential implications of antimicrobial resistance in this specific aquatic environment. This research is essential for developing strategies to mitigate the spread of antimicrobial resistance and safeguard public health in the region.

In this work, we use shotgun metagenomic to perform a quick description of the microbial composition of Llanquihue lake in three points of Puerto Varas city shore, aiming to characterize bacterial communities present in the beach, and identify the existence and abundance of ARGs in the environmental DNA due to the important role of these biologic elements for human health. This research will help to understand the hazards associated with AMR transfer from environmental reservoirs to humans by assessing the ARGs presented, and identifying the MGE which may drive ARGs transference. This works constitutes an example of how metagenomics can be useful in the surveillance of microbiological risks at city scale.

## 2. Results

### *2.1. Composition of Bacterial Communities That Inhabit Llanquihue Lake*

Shotgun metagenomic sequencing and bioinformatic analysis indicate a heterogeneous taxonomic composition of bacterial microorganism in the Llanquihue lake (Figure 1, Supplementary Figure 1 and Supplementary Table1), pointing the high diversity of bacterial microbial communities (Figure 2). Taxonomical assignation shows that the main phyla presented belong to Proteobacteria, Firmicutes, Bacteroidota, Actinobacteriota, and Verrucomicrobiota (Figure 2 and Supplementary Table 1). In the taxonomic data, we identify the presence of bacterial genera *Brucella*, *Mycoplasma*, *Mycobacterium*, *Microcystis*, *Leptospira* and *Flavobacterium*, which harbor bacterial species of interest in veterinary health [16]. In addition, we found the presence of bacterial genera belonging to the human

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intestinal microbiota such as *Prevotella*, *Coprococcus*, *Bifidobacterium*, *Faecalibacterium*, and *Ruminococcus* [17], in fact, *Prevotella copri* was one of the most abundant specie in the sample PV1.1. At specie level, we were able to identify a total of 3740 species (Supplementary Table 1), including environmental water related species such as *Nanopelagicus abundans* [18], *Fonsibacter ubiquis* [19], and *Planktophila vernalis* [20]. Interestingly, several taxonomical species related with skin and intestinal infections in humans were detected, mainly belonging to the genus *Campylobacte*r, *Clostridium*, *Escherichia*, *Mycobacterium*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus*, and *Yersinia* (Supplementary Figure 1). Although many of these taxonomic findings are repeated among the analyzed sites, the abundance of each of these taxonomies varies, indicating that microbial communities may be different regard its location (Figure 1).

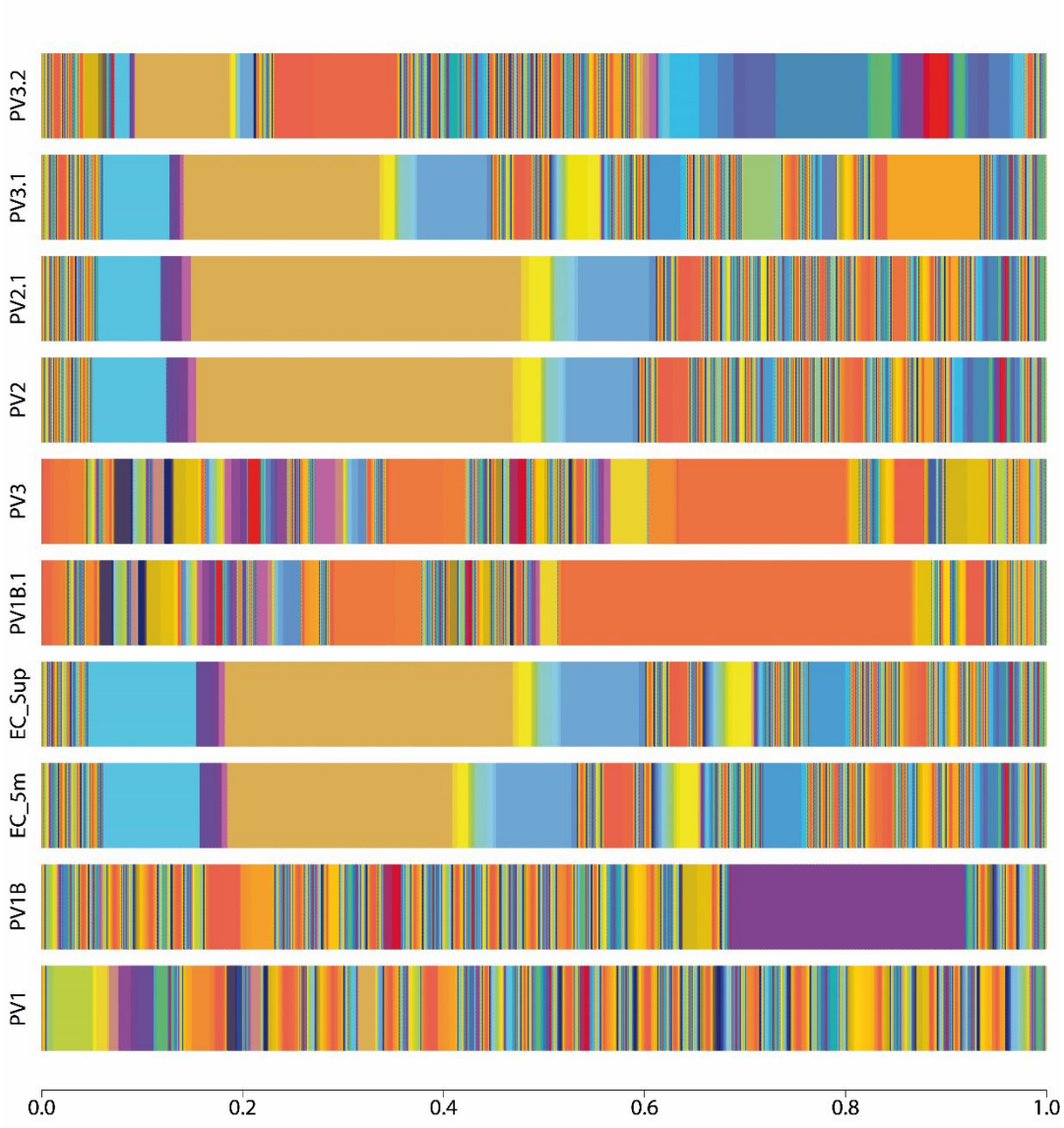


**Figure 1.** Taxonomy abundance at species level represented as stacked bar plot of each sample site of Puerto Varas shore. Three points of the Llanquihue lake beach were sampled (PV1, PV2, PV3) and one point (EC) 200 m far from the coast was sampled, at surface (SUP) and 5 meters submerged from the water column (5m). The coloration pattern of each bar depicts the percentage of abundance of each taxonomy, showing the microbial community structure.

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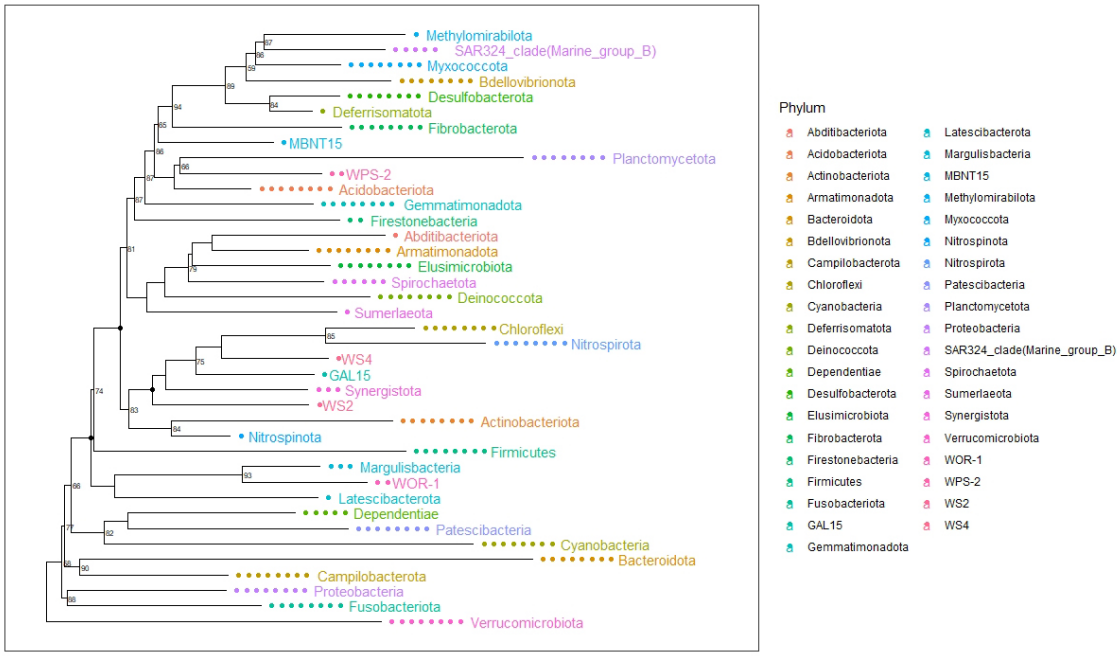


**Figure 2.** Phylogenetic trees reveal the intricate web of relationships among microbial phyla. Major phyla such as Verrumicrobiota, Fusobacteria, Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes emerge as prominent branches, highlighting their importance in processes such as fermentation and natural decomposition.

### *2.2. Identification of AMR Genes Present on DNA Recovered from Llanquihue Lake*

Our metagenomic analysis revealed a diverse array of antimicrobial resistance genes across the environmental DNA samples recovered from water. To report this, we group the different genes identified regarding their antimicrobial resistance family which belongs to (Figure 3). These included genes which confers resistance to antibiotics commonly used in human clinical treatments, such as β-lactams, chloramphenicol, and penicillin's, as well as genes associated with resistance to antibiotics used in agriculture and veterinary medicine such as macrolides, tetracyclines and fluoroquinolones, such as the tet genes, which confer resistance to tetracyclines by encoding for efflux proteins, or by encoding ribosomal protection proteins or enzymes that chemically modify tetracycline [21]. Another's remarkable families of genes with high presence were the bla family, which encode for the resistance to beta-lactam antibiotics [22,23], the dfr genes which encodes the trimethoprim resistant dihydrofolate reductase, initially founded in *Escherichia coli*, *Salmonella enterica* and *Pasteurella multocida* [24–26] and cat genes. This lately encodes for chloramphenicol acetyltransferase which inactivates chloramphenicol by addition of an acyl group [27]. Notably, the abundance and composition of ARGs varied spatially and temporally, with presence in areas impacted by anthropogenic activities, such as rainwater drainage and public beaches. Table 1 summarizes the antimicrobial family gene and the pharmaceutical drug example which confers resistance.

**Table 1.** Summary of antibiotic resistance genes found and associated resistance mechanisms.

| Family gen | Antibiotic family | Drug example | Resistance mechanism | References |
|---|---|---|---|---|
| bla | Betalactamics | Imipenem | Antibiotic Inactivation | [22] |
| cat | Phenicols | Chloramphenicol | Antibiotic Inactivation | [27] |
| cfx | Cefamycins | Cefoxitin | Antibiotic Inactivation | [28] |
| dfr | Diaminopyridines | Trimetoprim | Target modification | [24] |
| erm | Macrolides | Erythromycin | Target modification | [29] |
| Inu | Lincosamides | Clindamicin | Antibiotic Inactivation | [30] |
| mef | Macrolides | Erythromycin | Efflux Pump | [31] |

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| | | | | |
|---|---|---|---|---|
| msr | Macrolides | Erythromycin | Efflux Pump | [32] |
| oqx | Multi-Drug | Multi-Drug | Efflux Pump | [33] |
| tet | Tetracyclines | Doxicycline | Efflux Pump, Target Modification, Antibiotic Inactivation | [21] |
| vat | Streptogramins | Virginiamycin | Antibiotic Inactivation | [34] |

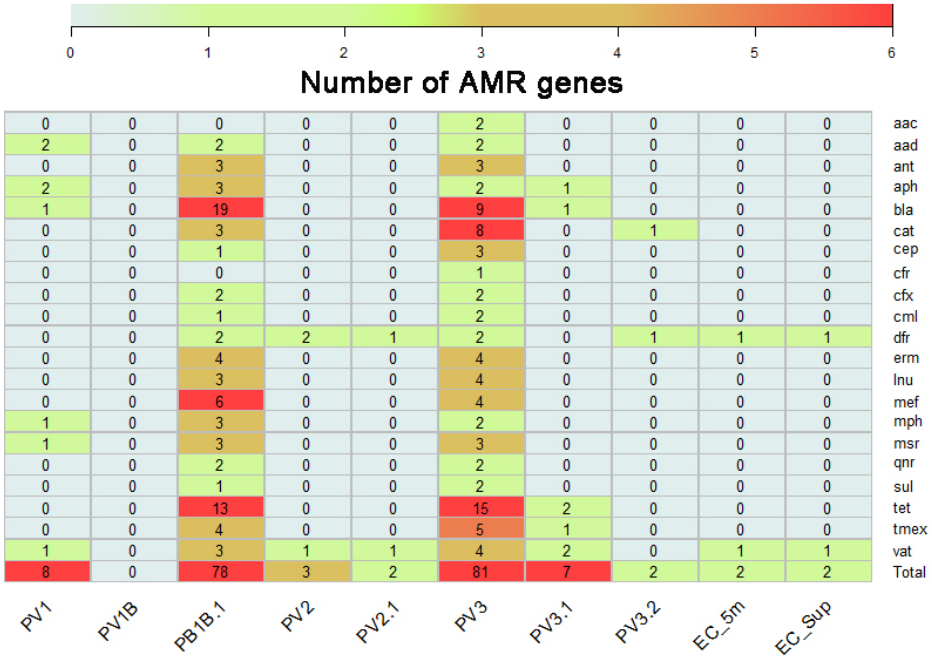


**Figure 3.** AMR genes identified on environmental DNA recovered from Llanquihue lake. The heatmap represents the genes identified grouped according to their antimicrobial resistance family which belongs to.

*2.3. Mobile Genetic Elements Carrying AMR Genes Are Related with Microbial Species of Health Interest*

To further completion of the characterization of ARGs present in the Llanquihue lake, we also screened metagenomic assemblies to explore the presence of MGE. The results showed the presence of mobile elements belonging mainly to the MOBP1 group, classification based on their relaxase gene [35], which included elements identified in bacterial host belonging to genus *Clostridiales*, *Clostridioides*, *Aeromonas*, *Vibrio*, *Enterococcus*, *Escherichia*, *Bacteroides*, *Parabacteroides*, *Klebsiella*, and *Proteus* (Supplementary Table 2). However, a significant portion of the mobile elements were not classified according to a MOB group, such as pR997, pSX2_LC6, pRIVM_C010068_1, pAFAEC, and pMMCAT_PdisCL06T03, identified originally in host such us *Proteus mirabilis*, *Shewanella sp.*, *Enterobacter hormaechei*, *Aliarcobacter faecis*, and *Parabacteroides distasonis*, respectively. Furthermore, we identified an overlap between the original host reported for MGE identified in our metagenomic data and the presence of harmful microbial species (Supplementary Figure 1 and Supplementary Table 2).

Because MGE plays a significant role in the evolution and adaptation of organisms by facilitating genetic diversity and horizontal gene transfer, we wondered what kind of genes are being carried by the plasmid sequences identified. For this purpose, we explore MGE sequences (Supplementary File 1), to look for the specific presence of ARGs in its genomic code. As a general trend, we found ARGs which confers resistance for erythromycin, azithromycin, lincomycin, doxycycline, tetracycline, amoxicillin, ampicillin, encodes mostly by the genes mph(E), msr(D), mef(A), erm(B), erm(F), lnu(C), tet(M), tet(W), tet(C), tet(O), blaSHV-12, and ant(6), respectively. These ARGs were carried in the

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MGE sequences (Supplementary File 1) identified initially in bacteria belonging to species such as *Lactococcus garvieae*, *Enterococcus faecalis*, *Acinetobacter sp.*, *Escherichia coli*, and *Shewanella sp*.

### *2.4. Antimicrobial Susceptibility Test of Microbial Isolates Do Not Show the Presence of Antimicrobial Resistance Patterns*

A total of 14 different isolates of enterobacteria were recovered and cultured from water samples. A total of 3 isolates belonging to *Citrobacter spp.*, 3 of *Enterobacter spp.*, 6 of *E. coli*, and 1 of *Rahnella aquatilis*, were identified by 16S rRNA PCR, Sanger sequencing, and BLAST. Six antibiotics were tested on all isolated bacteria, the inhibition halos obtained ranged from 15 to 49 mm. No resistant bacterial populations according to CLSI classification could be detected (Table 2).

**Table 2.** Susceptibility studies on bacteria isolated from Llanquihue lake.

| nº | Species | Antibiotic drug tested | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | cefotaxime | | Ampicillin / sulbactam | | Sulfamethoxosazole /trimethoprim | | gentamicin | | ciprofloxacin | | imipenem | |
| | | IZD(mm) | Int | IZD(mm) | Int | IZD(mm) | Int | IZD(mm) | Int | IZD(mm) | Int | IZD(mm) | Int |
| 23 | *Citrobacter freundii* | 36,3±0,6 | S | 19,7±0,6 | S | 26,0±1,0 | S | 19,3±0,6 | S | 39,0±1,0 | S | 28,3±1,5 | S |
| 55 | *Citrobacter gillenii* | 36,3±1,5 | S | 32,0±1,0 | S | 24,3±0,6 | S | 24,0±1,0 | S | 44,3±1,2 | S | 27,3±0,6 | S |
| 62 | *Citrobacter gillenii* | 32,7±1,2 | S | 38,0±2,0 | S | 22,7±0,6 | S | 20,7±0,6 | S | 50,0±1,0 | S | 37,0±1,7 | S |
| 2 | *Enterobacter absuriae* | 34,7±2,9 | S | 29,7±0,6 | S | 33,3±0,6 | S | 19,3±1,5 | S | 36,3±0,6 | S | 27,3±0,6 | S |
| 14 | *Enterobacter cloacae* | 35,7±1,2 | S | 30,0±0,0 | S | 32,7±1,2 | S | 19,7±1,2 | S | 40,3±0,6 | S | 31,0±1,0 | S |
| 39 | *Enterobacter ludwigii* | 34,0±1,7 | S | 32,3±1,2 | S | 31,3±0,6 | S | 23,3±0,6 | S | 47,3±0,6 | S | 33,0±1,0 | S |
| 41 | *Enterobacter ludwigii* | 35,0±2,0 | S | 34,7±0,6 | S | 35,7±0,6 | S | 24,0±0,0 | S | 49,0±1,0 | S | 35,3±1,2 | S |
| 21 | *Escherichia coli* | 36,7±0,6 | S | 19,7±0,6 | S | 24,7±0,6 | S | 24,3±0,6 | S | 34,0±0,0 | S | 31,0±1,0 | S |
| 22 | *Escherichia coli* | 35,3±06 | S | 20,3±1,2 | S | 26,3±0,6 | S | 20,3±0,6 | S | 41,3±1,2 | S | 31,3±1,5 | S |
| 26 | *Escherichia coli* | 37,3±0,6 | S | 23,3±0,6 | S | 28,3±2,1 | S | 22,0±1,0 | S | 38,3±0,0 | S | 29,3±1,5 | S |
| 27 | *Escherichia coli* | 34,0±1,0 | S | 20,0±0,0 | S | 27,0±1,0 | S | 19,7±2,1 | S | 35,3±0,6 | S | 31,3±1,5 | S |
| 28 | *Escherichia coli* | 33,0±0,0 | S | 21,3±1,5 | S | 27,3±0,6 | S | 23,7±0,6 | S | 41,0±1,0 | S | 32,3±1,5 | S |
| 42 | *Escherichia coli* | 36,7±1,5 | S | 20,3±1,2 | S | 24,7±0,6 | S | 19,0±1,0 | S | 38,3±0,6 | S | 30,3±2,3 | S |
| 3 | *Rahnella aquatilis* | 25,3±3,1 | S | 18,7±1,2 | S | 21,3±1,5 | S | 15,3±0,6 | S | 25,3±0,6 | S | 23,3±0,6 | S |
| ATCC 25922 | *Escherichia coli* | 31,3±0,6 | ✓ | 20,7±0,6 | ✓ | 24,7±0,6 | ✓ | 24,3±0,6 | ✓ | 45,0±0,0 | ✓ | 34,7±0,6 | ✓ |

IZD: Inhibition Zone Diameter expressed in millimeters Mean ± Standard Deviation, Int: Interpretation according to CLSI breakpoints [36], S: Susceptible, ✓: Quality control approved according to values defined by CLSI [36].

## 3. Discussion

Currently, studies with a One Health perspective are an urgent need due to threats associated with AMR. It is crucial to understand that this phenomenon is ubiquitous, so research must be conducted in humans, animals and the environment. Detection of a high abundance of ARGs in different environments corresponds to one of the first steps required to counteract this phenomenon. Nevertheless, as cultivable bacteria only represent a small fraction of the whole microbiota within a specific environment [37,38], the AR monitoring mostly depends on studios in total DNA extracts [39]. Studies related to ARGs in a variety of environmental compartments has been supported by molecular biology-based methods and sequencing methods [40–44]. Here, we studied the presence

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of ARGs in Llanquihue lake through metagenome sequencing. As metagenomics is a non-targeted method for detection and quantifying taxonomic and functional genetic diversity in each environment; these strategies allow to infer about occurrence and proportions of a variety of groups within a complex microbial community [45]. In addition, metagenomics is one of the most attractive tools to explore natural environments due to the large amount of information that can be obtained [46–48].

We could evidence the presence of cfxA6 and a cfxA2 genes in lake Llanquihue, related to the expression of class A beta lactamases, which have both cephalosporins and penicillins as substrates. In China, the presence of these genes related to this family has been detected also in different water bodies [49]. These genes were also found in wastewater from a treatment plant in Poland using metagenomics techniques [50]. In this study, we have found genes associated with the bla family: blaFAR-1, blaOXA-490 and blaOXA-491 and blaTEM-102 and -104, these genes are also associated with the expression of beta-lactamases in different pathogenic bacteria. Regarding blaFAR-1, blaOXA-490 and -491, we did not find reports of their presence in water bodies, but we did find reports of their presence in blaTEM-102 and -104. These genes have been reported in different bodies of water, and these studies mention the potential risk to human health of these genes being found in the environment [51–53].

Interestingly, we were also able to demonstrate the presence of different genes associated with the mcr gene family (mcr-3, -4, -5 and -7). These genes are related to colistin resistance, a highly relevant drug in the treatment of infections complicated by multiresistant gram-negative bacteria [54–56]. Some studies demonstrate the presence of these genes in water bodies around the world [57,58]. This gene's presence in a lake where recreational activities are done constitutes a risk for the population. Other of the most abundant gene families found in Lake Llanquihue were genes associated with resistance to tetracyclines such as tet(37), tet(A), tet(C), tet(O), tet(Q) and tet(W). Several studies have shown the presence of these resistance genes in aquatic environments [59–62]. For example, tet(37) gene family, has been reported at the environmental level in an anthropogenically stressed estuary on the northwest coast of Portugal [63]. In particular, the presence of these genes poses risks for productive activities associated with aquaculture. This is because one of the most widely used antibiotics in freshwater production cycles is oxytetracycline [64]. The potential expression of these genes in pathogenic bacteria affecting farmed fish could cause the ineffectiveness of these treatments. In summary, the presence of these genes in DNA isolated from water reservoirs highlights the widespread distribution of antimicrobial resistance determinants in the environment. Additionally, our analysis unveiled the presence of MGE, such as plasmids associated with ARGs, highlighting the dynamic nature of AMR in aquatic ecosystems and its potential dissemination.

One limitation of our study was to isolate and characterize bacterial strains with antimicrobial resistance phenomena. As detection of the presence of ARGs from data obtained by sequencing does not necessarily imply the expression of such genes in each microbial community, further empirical tests are required for describing putative expression of resistant phenotypes [65]. To aboard this, we performed susceptibility assays in 20 microbial isolates obtained from Llanquihue lake. Although in our microbiologic results the presence of multiresistant strains was not detected as was expected regard to the metagenomic results presented here, these results might not be entirely representative of the occurrence of multiresistant strains in the environment. ARGs detection through metagenome sequencing gives information about both, culturable and unculturable bacteria, thus classic microbiological techniques for culture and susceptibility assays from microbial isolated have limitations that should be considered.

Proper watershed management has important positive effects on the mitigation of human health risks associated with the presence of ARG in the environment [66]. The implementation of effective public politics on water management, such as regulating and monitoring the discharges of domestic, industrial and hospital wastewater into water bodies, can help to avoid ARG transference and pharmaceutical dispersion [67,68], for example, reducing the use of agricultural antibiotics close to water bodies [69] or limiting the presence of aquaculture activities in fresh water [70], would

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contribute to reducing the load of pharmaceutical pollutants in water systems. Additionally, constant monitoring of water quality would contribute significantly to decision making in promoting sustainable practices in the watershed [71]. Moreover, evidence-based decision-making on water quality, supported by monitoring data, can be crucial for delivering safe drinking water, optimizing water quality, and managing water resources effectively [72,73]. By minimizing water pollution, selective pressure on harmful microorganisms that inhabit the aquatic environment would be reduced, diminishing the spread of antimicrobial resistance and virulence genomic elements [74,75]. This comprehensive approach would not only protect the health of local communities by safeguarding the purity of the water resource but would also contribute to the preservation of antibiotic effectiveness and sustainable public health management in the long term [76].

## 4. Materials and Methods

*Sample Collection and Microbial Isolation*

We collected 10 water samples from the coastal shore of Puerto Varas city (Supplementary Figure 2). 3 liters for each sample site were collected using sterile 1 L glass bottles and preserved with icepack until they were processed within the same day they were taken. A total of 3 liters of water were filtered through mixed cellulose ester (MCE) membranes of 0.22 μm pore size (Merck-Millipore #GSWP04700, USA), using a glass filter system pumped with negative pressure. Filters were stored in RNA Later (Sigma-Aldrich #R0901, USA), until DNA extraction as described below. In parallel, 1 mL of water was streaking on BHI, TSA, EMB, Miller-Hinton, and McConkey agar plates, and cultivated at 25° C for 24 h. Cell colonies obtained were passed 2 times to facilitate its purification in agar plates and then, Gram stain was used to check the purity of the isolated bacteria. Isolated microorganisms were observed under microscopy and stored in sterile glycerol 10% v/v at -80 °C.

*DNA Purification and Metagenomic Sequencing*

The stored MCE filters were used for DNA extraction employing AccuPrep Genomic DNA Extraction Kit (Bioneer #K-3032, Korea), following manufacturer instructions. Briefly, filters were resuspended in 500 μl DNA Extraction buffer and stirred to release microbial cells. An enzymatic digestion with 20 μl of lysozyme (20 mg/mL) and 20 μl of proteinase K (20 mg/mL) were used to disrupt microbial cells. The suspension was incubated for 1 h at 37 °C and then for 1 h at 55 °C. After enzymatic digestion, we followed the steps provided by the manufacturer for bacterial DNA extraction. The quality of obtained the DNA was checked by 1% agarose gel electrophoresis, while DNA quantity was measured by absorbance, and the ratios 260/280 nm were calculated to assess the purity of DNA obtained. Before DNA sequencing, we test the amplification capacity of DNA using 16S bacterial universal PCR. A total of 1 μg of DNA was sent to Novogene (USA) genomic service for shotgun metagenomic sequencing. DNA was sequenced by paired-end (2x150 bp) reads using the Illumina NovaSeq 6000 platform with an output of 6 GB per sample.

*Metagenomic Data Analysis and Identification of AMR Genes*

Raw data obtained from the sequencing provider was initially inspected with FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc), and then reads were filtered and trimmed using Trimmomatic [77] using the following parameters: LEADING:20, TRAILING:20, SLIDINGWINDOW:5:20, AVGQUAL:20, and MINLEN:90, followed by the application of Bowtie2 to screen out the contaminant DNA sequences from human and viruses [78]. The paired-end files were merged using the script provided in the Microbiome Helper v2.3 pipeline [79] and metagenomic data were procesed to obtain metagenomics de novo assembly using MegaHit [80] and the quality of conting obtained were inspected using Quast [81]. The taxonomic profiling was obtained at specie level using Kraken2 [82], keeping the taxonomic assignation with over 500 hits by sample, while antimicrobial resistance genes were inspected using ABRicate [83], utilizing the Resfinder [84] databases. Mobile genetic elements were retrieved using plaSquid [85]. The fasta files obtained from plaSquid were used to look for the presence of AMR genes carried in the mobile elements using

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ABRicate as described above. Data obtained was imported to R statistical language [86] for further analysis and representation using phyloseq [87] and ggplot2 [88] packages.

*Antimicrobial Susceptibility Assay*

Antimicrobial susceptibility testing of 14 isolates was performed using the disk diffusion method described by Hudzicki, 2009 [89]. Mueller-Hinton I agar (DIFCO) was employed to evaluate bacterial susceptibility to six antibiotics drugs: ampicillin (2 µg), imipenem (10 µg), norfloxacin (10 µg), gentamicin (30 µg), vancomycin (5 µg), erythromycin (15 µg), tetracycline (30 µg) and trimethoprim-sulfamethoxazole (1.25–23.75 µg). Zone inhibition diameters were interpreted according to CLSI breakpoint tables [90]. All studies were done in triplicate. The halo measurements were expressed as the average of the measurements plus the standard deviation. *Eschericha coli* ATCCÓ 25922 was used as a quality control strain.

## 5. Conclusions

Our study provides valuable insights into the prevalence, diversity, and nature of antimicrobial resistance genes presented in environmental water recovered from a lake system enclosed beside a city. By elucidating the dynamics of ARG and virulence genes dissemination, we contribute to the collective efforts aimed at combatting the occurrence of resistance phenoms and preserving the efficacy of antimicrobial agents for future generations. Research on the identification of antimicrobial resistance and virulence genes in environmental water highlights the urgent need for standardized monitoring methods to address the global public health threat posed by antibiotic resistance. Understanding the presence, diversity, and transmission pathways of resistance genes in water environments is essential for developing effective strategies to mitigate the spread of antimicrobial resistance between microbial species and the generation of antimicrobial multidrug resistant microorganism.

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**Data Availability Statement:** The raw data produced from DNA sequencing in this study was deposited at the ENA-EMBL database under the accession number PRJEB76156. Metadata and metagenomic tables obtained from bioinformatics analysis can be found in Supplementary Files.

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