Emerging Contaminants 12 (2026) 100723

Contents lists available at ScienceDirect

Emerging Contaminants

journal homepage: www.keaipublishing.com/en/journals/emerging-contaminants

Hydrogeochemical drivers of antimicrobial resistance (AMR) in the Maipo River basin, Chile: The role of treated wastewater effluent

George J.L. Wilson [a], Ismael Casado [b], Daren C. Gooddy [c,d], Linda Daniele [b,e], Laura A. Richards [a,*]

[a] Department of Earth and Environmental Sciences and Williamson Research Centre for Molecular Environmental Science, The University of Manchester, Manchester, M13 9PL, United Kingdom
[b] Department of Geology, Faculty of Physical and Mathematical Sciences, University of Chile, Santiago, Chile
[c] UK Centre for Ecology & Hydrology (UKCEH), Maclean Building, Wallingford, Oxfordshire, OX10 8BB, United Kingdom
[d] Lancaster Environment Centre, Lancaster University, Lancaster, United Kingdom
[e] Centro de Excelencia en Geotermia de los Andes (CEGA), Faculty of Physical and Mathematical Sciences, University of Chile, Santiago, Chile

ARTICLE INFO

Article history:
Received 8 April 2026
Received in revised form
2 July 2026
Accepted 15 August 2026
Available online 18 August 2026

Keywords:
River water quality
Antibiotic resistance
ARGs
Treatment plants
Surface water
South America
Chemical mixtures

ABSTRACT

Antimicrobial resistance remains an imminent and serious public health challenge. Wastewater presents an important reservoir for antimicrobial resistance evolution and transmission in the environment. Here, we investigated antimicrobial resistance and water quality markers in the Maipo River basin, Chile, in a ~120 km longitudinal study of predominantly surface water ($n = 13$) in Santiago Metropolitan Region. Pollution indicators (*e.g.* NO_3^-, dissolved organic carbon (DOC), tryptophan-like fluorescence, IS*1247*, 16S rRNA, *merA* and *crAss64*) increased from upstream to downstream in the Mapocho and Maipo rivers and particularly downstream from Santiago and/or following wastewater discharge. There was no detectable AMR in sampled groundwater ($n = 2$; 50–120 m) nearby to the wastewater treatment plant, plausibly because the sampled wells represent relatively evolved groundwater with longer residence times, compared to river water. Wastewater tracers (Tryp-like fluorescence, log(16S rRNA), DOC, *crAss64*, PO_4) indicated strong associations with IS*1247* ($p < 0.01$), whilst DOC, PO_4, and log(16S rRNA) were associated with *sul1* ($p = < 0.05$). Results suggest mobile genetic elements (MGEs) such as insertion sequences may be promoted by, or enriched in, treated wastewater. These findings improve our understanding of how treated wastewater affects downstream water quality in populated river basins.

1. Introduction

Recent reports estimate that antimicrobial resistance (AMR) could be attributed to 1.9 million deaths in 2050 [1]. The rapid spread of AMR poses severe challenges for public health, agriculture, and the environment, particularly so in Latin America, where surveillance, transmission and remedial studies are currently sparse and/or inadequate, often due to limited resources and/or laboratory capabilities [2].

Pathogens with drug-resistant properties (*i.e.* AMR) are a problematic occurrence for treating disease [3]. Environmental microbiomes are known to harbour a large diversity of genes that could plausibly contribute to the increase of drug-resistant bacteria [4]. Yet, there is a relative lack of research on the existence of AMR within environmental reservoirs and particularly the effects of anthropogenic activities on surface water and groundwater [5,6]. Whilst Karkman et al. (2019) and Yu et al. [7] propose that subinhibitory levels of antimicrobials result in minimal selection pressure for AMR genes, Andersson and Hughes [8,9] provide evidence to the contrary, highlighting the importance of increasing the evidence base around AMR in environmental reservoirs and its potential implications.

In addition to antimicrobials, some water quality parameters are thought to impact and/or contribute to AMR dissemination, such that antimicrobial concentrations and reported AMR often

* Corresponding author.
E-mail address: laura.richards@manchester.ac.uk (L.A. Richards).
Peer review under the responsibility of Editorial Office of Emerging Contaminants

https://doi.org/10.1016/j.emcon.2026.100723

show disparities [10,11]. It is recognised that some metals (*e.g.* Zn and Cu), antibacterial biocides and micro/nanoplastics may co-select for AMR [12–16], whilst other (bio)geochemical factors (*e.g.* temperature, nutrients and pH) may increase AMR prevalence by mediating stress-induced horizontal gene transfer (HGT [17–21]).

Wastewater treatment plants (WWTPs) are often ineffective at completely removing AMR-bearing pathogens [10,22–28] and water/wastewater treatment processes, arguably, provide suitable conditions for AMR selection [6,29–31]. Wastewater is often considered a potential hotspot for AMR dissemination because of high diversities of chemical and microbial pollutants which may be conducive to HGT. Whilst WWTPs may reduce absolute concentrations of AMR genes [32], high AMR richness and a high prevalence of mobile genetic elements (MGEs) such as insertion sequences (ISs) have been found in wastewater effluents, compared to less human-impacted environments [30,33].

Tracking relationships between wastewater and AMR in freshwater environments provides an important focal point of research in the study of AMR dissemination. Tracers of wastewater, such as nutrients, organic carbon and tryptophan-like fluorescence [34] and their relationships to AMR prevalence have been investigated to a limited extent [11]. Recently, the use of human gut bacteriophage markers (*e.g. crAss64*) have been used for microbial source tracking (MST) to identify human-derived faecal pollution [35,36]. Since *crAss*-like phages are not linked to integrons, these may provide useful indicators of pollution-AMR relationships as they are less likely to be co-selected with AMR-related genes [37].

Critical gaps remain on longitudinal transport of AMR genes in rivers and streams following WWTP discharge [30]. Furthermore, studies on AMR prevalence and transmission in wastewater and receiving waters may be of particular interest to public health in regions where wastewater is used for irrigation (*e.g.* Santiago [6,38,39]). In Chile, whilst a National Action Plan against AMR has been developed, the role of WWTPs in AMR dissemination may be overlooked [40–42]. Thus, the aim of this pilot study was to quantify the influence of wastewater effluent on water resources of the River Maipo basin in Chile. This was achieved by quantifying selected water quality and (bio)geochemical parameters, including AMR, in i) surface water from upstream and downstream samples in the Maipo River basin ii) groundwater and iii) wastewater influent/effluent of WWTP. Additionally, this study aims to quantify associations between key AMR-related markers and wastewater tracers to estimate the potential effect of wastewater on AMR prevalence in surface waters in Chile.

2. Methods

2.1. Site description

The Maipo River basin in Chile spans 250 km from its meltwater source high in the Andes to the Pacific Ocean, south of the port of San Antonio (Fig. 1a). The Maipo River provides the major source of irrigation and drinking water to the Santiago Metropolitan Region, supplying a population of 7.4 million [43]. Municipal wastewater in Santiago is predominately treated at La Farfana wastewater treatment plant (WWTP; Fig. 1b), with a capacity of 8.8 m^3/s ([44].). The confluence of the Mapocho and Maipo rivers lies southwest of the city, although mixing between the two rivers occurs prior to the confluence. For example, the SE-NW flowing Canal San Carlos diverts water from the Maipo River into the Mapocho River. The Maipo River basin hosts large reserves of copper-iron-molybdenum porphyry deposits [45], which may increase dissolved metal and SO_4^{2-} concentrations [46,47]. River water sampling sites ($n = 11$) were selected to represent a broad range of influence from wastewater, whilst incorporating a suitable spatial coverage (distributed ~ 20 km apart) and accessibility of sites. The selection of groundwater sites ($n = 2$) was based on the availability of boreholes within the vicinity (~3 km) of the WWTP.

2.2. Surface water and groundwater sampling

River water ($n = 11$), groundwater ($n = 2$) and wastewater influent/effluent ($n = 2$) were sampled from the Maipo River drainage basin (Fig. 1) in August 2024. Near-bank river water samples were sampled from the Mapocho River ($n = 6$), the Maipo River ($n = 4$) and an upstream tributary (Estero El Sauce; $n = 1$). Groundwater samples ($n = 2$) were from private boreholes (50 m and 120 m depth) and were flushed prior to sampling, to remove standing water from the pipes.

Water samples for the analysis for major/minor elements and dissolved organic carbon (DOC) were filtered (0.45 μm; Sartorius Minisart® regenerated cellulose) into glass bottles which were sealed with Parafilm M tape. Prior to fieldwork, unused glass bottles were bathed in 10 % nitric acid for 24 h, rinsed copiously with ultrapure water and then baked (450 °C). Except during transit, water samples were stored at 4 °C until analysis and/or further processing.

A handheld meter (Myron L® Ultrameter II™) was used to measure pH, electrical conductivity (EC) and oxidation reduction potential (ORP) in the field. The instrument was regularly three-point calibrated using standard solutions (HI-6031, Certipur ® pH 4.01, 7.00 and 10.00).

Environmental DNA (eDNA) samples were collected in sterile 2 L high-density polyethylene bottles and transported to the University of Chile, prior to filtration. Filtration took place at the end of each sampling day with a method previously described in Wilson et al. [11]. Samples were filtered (250–500 mL; Millipore Express™ PLUS 0.22 μm) in triplicate; filter papers were stored at −20 °C until analysis. Samples were transported from Chile to the UK using a pre-chilled vacuum flask containing an icepack. For analysis of eDNA, filter papers were transported on dry ice from Manchester to Resistomap Oy, Helsinki, Finland, in December 2024.

2.3. Quantification of target gene sequences

2.3.1. DNA extraction

The DNA was extracted using the DNeasy PowerWater Kit (QIAGEN) at Resistomap Oy, according to the manufacturer's protocol. The final eluted volume of DNA was 50 μL. To ensure that the DNA extraction was satisfactory, the quality and quantity of the DNA was measured using a NanoDrop spectrophotometer and Qubit fluorometer, respectively.

2.3.2. High-throughput smartchip qPCR (HT-qPCR)

AMR genes, MGEs, taxonomic and pathogen markers were quantified using High-Throughput Smartchip qPCR (HT-qPCR) at Resistomap Oy. The SmartChip MultiSample Nanodispenser filled 5184 reaction wells with SmartChip TB Green Expression Master Mix, 20 nL of template DNA, 300 nM of forward and reverse primers, nuclease-free water and 1 % glycerol. The qPCR conditions included an initial denaturation step from 95 °C for 10 min, 40 cycles with denaturation at 95 °C for 15 s, annealing at 60 °C for 60 s. To verify the specificity of amplification, melt curve analysis was performed following the completion of the cycling, with an increase to 97 °C at 0.4 °C/step.

The selection of 36 target sequences was based on i) AMR-

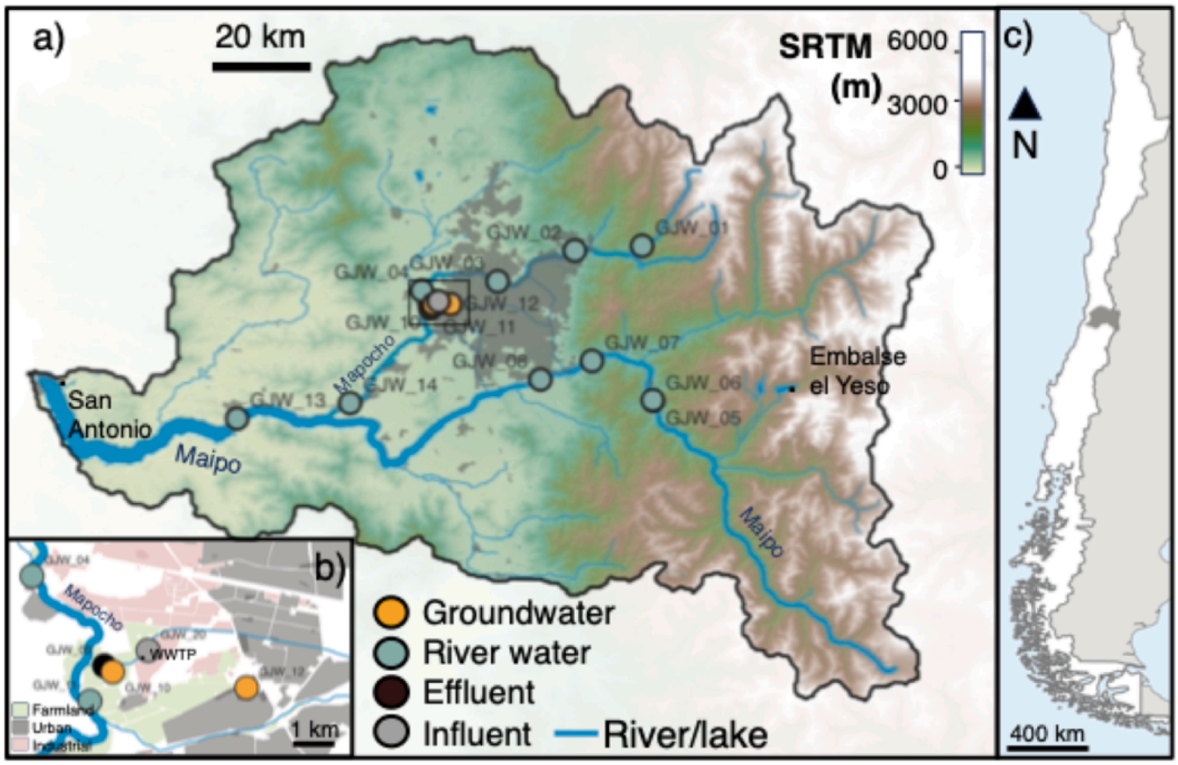

Fig. 1. River water ($n = 11$), groundwater ($n = 2$) and wastewater influent ($n = 1$) and effluent ($n = 1$) was sampled to understand longitudinal changes in AMR-related water quality in a) the Maipo River drainage basin as delineated (black border) using the HydroBASINS dataset [48], b) the area surrounding La Farfana wastewater treatment plant (WWTP), noting the coordinates of site GJW_12 have been rounded for confidentiality and c) Santiago Metropolitan Region, Chile. Elevation was derived from the NASA Shuttle Radar Topography Mission [49]. Land use and water features were obtained from OpenStreetMap under an Open Database License [50].

related clinical priorities in Latin America [1], ii) the WHO Bacterial Priority List (2024), iii) comparison to results obtained in prior studies of the authors (*e.g.* Wilson et al. [11]), iv) representation of a broad range of AMR, MGEs and related resistance markers and v) based on advice from Resistomap, including the *96-Genes Panel for One Health* list [51]. The target gene assay was optimised to maximise the chance of gene detection (noting that a range of gene abundances would be expected across the sample set), using information from a global surface water database, provided by Resistomap. The 36 targeted genes were: *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, *intI1*, IS*1247*, *tnpA*, IS*26*, *IncP oriT*, Tn*5403*, *vanA*, *vanB*, *sul1*, *folP*, *merA*, *mcr-1*, *acrB*, *mdtA*, *tetM*, *tetW*, *tetA*, *aadA*, *rmtB*, *crAss64*, *qnrS2*, *qnrB*, *catIII*, *ermB*, *Bacteroides* spp., *Acinetobacter baumannii*, *Escherichia coli uidA*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Staphylococcus* spp., *Enterococcus* spp. and 16S rRNA. To normalise the abundance of genes to an estimation of the total bacteria population, genes were reported relative to 16S rRNA gene abundance – subsequently referred to in this manuscript as gene prevalence.

2.3.3. Data processing

Examination of melt curves and qPCR efficiency was used to ensure high quality data. Amplicons with unspecific melt curves and/or yielding multiple peaks were considered to be false positives and were discarded from the analysis. Only values smaller or equal to the threshold cycle ($C_T = 27$) were considered acceptable for detection [52,53]. Additionally, reactions with qPCR efficiency outside the range 1.7 – 2.3, as calculated from the standard curve, were excluded from the analysis. Furthermore, if the technical replicate ($n = 3$) standard deviation exceeded 0.5, the replicate with the largest deviation was discarded. If three technical replicates remained following this step, a replicate with an efficiency furthest from 1.9 to 2.1 was excluded from the analysis. For each sample tested, the mean C_T ($n = 2$ or 3) of technical replicates was used to calculate ΔC_T values. If genes were detected in only one of the three technical replicates, the results were excluded. The $2^{-\Delta CT}$ method ($\Delta C_T = C_{T,\ Target} - C_{T,\ 16S\ rRNA}$) was used to calculate relative gene abundance, in relation to the 16 S rRNA gene [54].

2.4. Geochemical quantification

2.4.1. Major and trace inorganic analytes

Geochemical analysis was undertaken at the Manchester Analytical Geochemistry Unit (MAGU) facility at The University of Manchester. Analytes SO_4^{2-}, PO_4, NO_3^-, NO_2^-, Cl^-, Br^- were quantified with a Dionex ICS-5000 Ion Chromatography (IC) System with Dionex IonPac AS18 columns. Analytes Cr, Pb, Mn, Cu, Zn and As were analysed with mass spectrometry (ICP-MS, Agilent 7500cx). To ensure analytes remained dissolved in solution prior to nebulisation, ICP samples were acidified to 2 % HNO_3 (Aristar®, VWR Chemicals BDH®, UK). Multi-element Certified Reference Materials (CRMs; ICM1-500, LGC6020, LGC6026 and LGC6027; LGC Standards, UK) were used to verify the accuracy of the results. For the purpose of calculating of the Cl/Br molar ratio, in instances of non-detects in Br, the concentration of Br was set at half of the Hubaux-Vos detection limit (LoD = 0.0243 mg/L).

2.4.2. DOC quantification

DOC was quantified with a Shimadzu® Total Organic Carbon Analyser (TOC-VCPN; ASI-V autosampler) using the *TC–TIC* method; CRM QC1388 (Sigma-Aldrich, UK) was used to verify the

accuracy of the results.

2.4.3. Excitation-emission matrix (EEM) fluorescence spectroscopy

To establish the characteristics of the quantified DOC, excitation-emission matrix (EEM) fluorescence spectroscopy was utilised. As an indicator for wastewater-derived contamination, tryptophan-like (Tryp-like) fluorescence [55] was quantified using a Varian Cary Eclipse fluorescence spectrophotometer at the British Geological Survey, Wallingford. Data was processed using an R script adapted from Lapworth and Kinniburgh [56]. Methods for fluorescence spectroscopy including absorbance correction have been published previously [57,58].

2.5. Statistical analysis

All statistics and data analysis was undertaken in R (4.3.2) in the RStudio (2024.12.0 + 467) environment [59]. The ComplexHeatmap package in R [59,60] was used for heatmap analysis on normalised and centred values. Euclidean hierarchical clustering was used to identify similarity between variables and individual samples [60,61]. A Wilcoxon signed-rank statistic was computed for paired comparisons of non-parametric nature, as tested by the Shapiro-Wilk test, reported as "*V = V-statistic, p = p=value*", where the *V*-statistic is the sum of the positive signed ranks. Confidence intervals were reported at $p = 0.05$.

3. Results and discussion

3.1. Data quality

The CRM bias for DOC was 1.8 % (QC1388). The mean CRM bias for Cl^-, Br^-, NO_3^-, SO_4^{2-} and PO_4 was 7.7 %, 6.7 %, 2.2 % 10.7 % and 6.0 % (LGC6020 and VHG-ICM1), whilst the repeat analytical error ($n = 3$) was 0.4 %, 18.6 %. 2.8 %, 1.1 % and 0.1 %, respectively. The CRM bias for Fe, Mn, Cu, Zn and As was 14.3 %, 20.4 %, 12.0 %, 10.4 % and 11.9 % (CRM6026 and CRM6027) whilst the repeat analytical error ($n = 3$) was 5.4 %, 27.4 %, 3.6 %, 33.3 % and 5.2 %, respectively. The repeat error for HT-qPCR relative abundance was as follows: *intI1* (19.6 %), *tnpA* (23.2 %), *vanA* (15.7 %), *sul1* (6.5 %), IS*1247* (15.2 %) and *crAss64* (7.6 %).

3.2. Surface water and groundwater characteristics

Physicochemical, organic and AMR parameters were measured in water samples in the Maipo River basin. Water characteristics of river samples were as follows: pH 7.1 – 8.5; 127 – 1580 µS/cm EC; 6.3 – 14.3 °C; 0 – 5.1 mg/L DOC; 2 – 23 mg/L NO_3^- and 5 – 323 mg/L SO_4^{2-} (Fig. 2 and Supplementary Fig. 1). High EC, compared to previous studies in the Maipo basin, may be explained by strong interactions with evaporites, carbonates, andesites and granodiorites coupled with drought conditions at the time of sampling [62]. Groundwater ($n = 2$) was oxidised (230 – 460 mV ORP), brackish (1230 – 1880 µS/cm EC) and low in DOC (0 – 0.4 mg/L). High NO_3^- (50 – 66 mg/L) was consistent with previous observations in Santiago Metropolitan Region groundwater [63].

3.3. Water parameters displayed prominent change from upstream to downstream

Selected water parameters were characterised to understand longitudinal hydrogeochemical changes in the Maipo River basin. The Maipo and Mapocho displayed distinct water characteristics – headwaters of the Mapocho were characterised by lower Cl/Br, EC, SO_4^{2-}, compared to headwaters of the Maipo (Fig. 2 and Supplementary Fig. 1). High Mn, Cu and Zn concentrations were detected upstream in the Mapocho and at site GJW_01 in

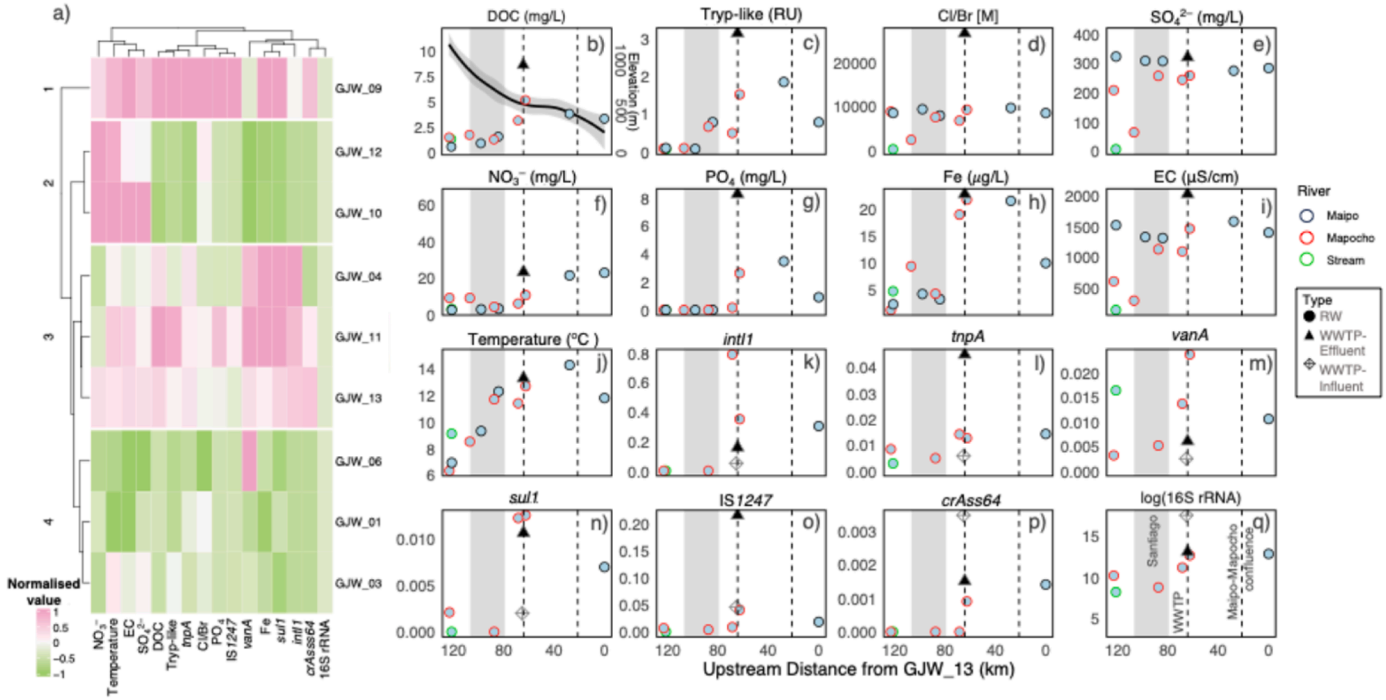

Fig. 2. Characteristics of water in the Maipo River basin; a) heatmap analysis of normalised and centred parameters reveals water quality was similar amongst four groups: 1 – WWTP effluent, 2 – groundwater, 3 – urban-influenced river water and 4 – pre-urban-influenced river water, b–q) longitudinal trends in water quality, whereby upstream distance refers to distance from site GJW_13 (Fig. 1). An elevation profile is shown in plot b (SRTM, 2013). Gene prevalence reported relative to 16S rRNA, whilst 16S rRNA is reported in copies/mL. Santiago Metropolitan Region is represented by the grey box; WWTP discharge is shown by the dashed line in the centre of the sub-plots and the confluence of the Maipo and Mapocho rivers is shown by the dashed line on the right.

particular – 1174 μg/L, 549 μg/L, 437 μg/L, respectively (Supplementary Fig. 1c–e), which was consistent with previous regional characterisation studies [47]. Otherwise, most indicators of pollution (*e.g.* NO_3^-, DOC, Tryp-like, IS*1247*, 16S rRNA, *merA* and *crAss64*) increased from upstream to downstream in the Mapocho and Maipo rivers and particularly downstream from Santiago and/or post-WWTP discharge. The longitudinal gradients observed in EC, SO_4^{2-} and NO_3^- likely reflect a combination of natural hydrogeochemical evolution and anthropogenic inputs along the basin. Upstream waters draining the Andean catchment interact with volcanic and carbonate lithologies, which may partly explain elevated SO_4^{2-} and EC in the Maipo headwaters [62,64]. Further downstream, increases in DOC, NO_3^- and microbial markers coincide with urban influence and wastewater discharge from Santiago. These patterns are consistent with progressive mixing between relatively dilute mountain waters and increasingly anthropogenically influenced river water along the basin.

Spearman rank testing yielded no significant correlation with any AMR gene and downstream distance ($p < 0.05$), noting that the limited sample number impeded statistical testing. Whilst several genes (*e.g. vanB*) were detected upstream from Santiago and in fact *folP* was highest in Mapocho headwaters (Supplementary Fig. 1n), others (*e.g. merA*, *acrB*, *tetM*, *tetW*, *tetA* were not detected prior to the Santiago Metropolitan Region. Interestingly, Andersson et al. [65] detected *tetM* and *tetA* in both agricultural and urban stretches of the Maipo Basin. The detection of IS*26* and *IncP_oriT* occurred only after human settlement but before the influence of WWTP discharge – suggesting that WWTP effluent was not the sole driver of MGE occurrence in the Maipo basin.

Whilst the abundance of 16S rRNA in the untreated wastewater (4.3×10^7 copies/mL) was two orders of magnitude higher than the effluent ($4.9\times\ 10^5$ copies/mL), in contrast, the prevalence of AMR genes in the treated effluent was significantly higher (by a factor of 3.6 ± 1.4) compared to WWTP influent ($V = 256$, $p = 0.01$, n-paired $= 27$). Whilst this presents important preliminary findings that are consistent with elsewhere in Chilean rivers [23], it does not necessarily provide evidence for WWTP-induced selection, noting i) statistical limitations of such a small sample number and ii) prominent longitudinal changes to bacteria and phage communities in the Maipo River [65], as this will likely affect MGE and/or AMR prevalence [7].

3.4. Wastewater tracers indicated strong associations with IS1247 and modest association with sul1

As a mediator of AMR gene mobilisation, IS*1247* was measured and compared to six indicators of wastewater contamination (Fig. 3). The relationship between bulk microbial proxies, particularly Tryp-like fluorescence and log(16S rRNA) with IS*1247* ($r_s = 0.83$ and 0.96; $p < 0.01$, respectively) suggests that higher density of microorganisms promotes cell-to-cell contact and thus the probability of genetic exchange via HGT [4]. As there was correlation between IS*1247* and other wastewater indicators (Fig. 3; $p < 0.05$), co-variance may be influenced by numerous factors that favour HGT and IS prevalence [33,66], including antibiotics [8,9] and metals [67,68], though noting the significant

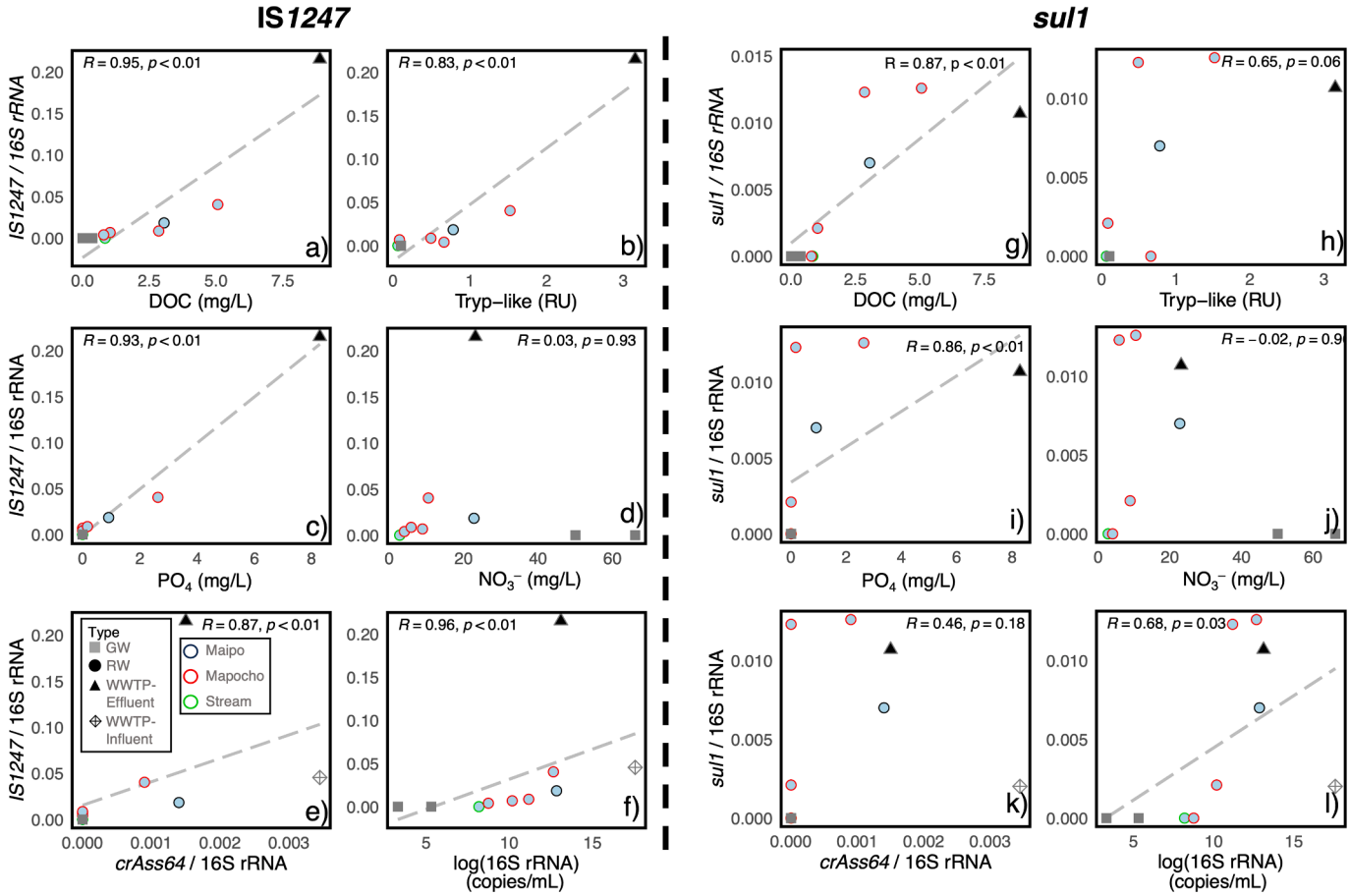

Fig. 3. Wastewater indicators DOC, Tryp-like, PO_4, NO_3^-, *crAss64* and log(16S rRNA) were compared to a–f) IS*1247* and g–l) *sul1* in the Maipo River basin. Spearman rank coefficient (r_s) with corresponding p-value is displayed.

downstream temperature gradient may also influence microbial activity in the Maipo Basin (Fig. 2j [69]).

Whilst IS*1247* demonstrated a correlation with *crAss64* prevalence ($r_s = 0.87$), the relationship was weaker than say PO_4 and DOC ($r_s = 0.93$ and 0.95, respectively), suggesting that human-derived faecal pollution could explain a large proportion of IS occurrence [36], with plausibly another input (*e.g.* agricultural run-off [18,65]), perhaps through *in-situ* selective pressure [8,66]. Given the absence of high IS*1247* in groundwater elevated in NO_3^-, this may indicate that nutrients (or at least NO_3^-) do(es) not solely initiate *in-situ* promotion of HGT as suggested in Taihu Lake, China [21].

Similarly, wastewater tracers were tested for co-variance with *sul1*, an AMR gene often located on class 1 integrons [70]. Spearman rank testing revealed statistical correlation with DOC, PO_4, and log(16S rRNA; $r_s = 0.87$, 0.86 and 0.68, respectively), but not to *crAss64* prevalence, Tryp-like fluorescence nor NO_3^- ($r_s = 0.46$, 0.65 and −0.02, respectively; Fig. 3). Whilst genes *sul1* and IS1247 were correlated ($r_s = 0.77$; $p = 0.01$), stronger association between IS1247 and wastewater tracers suggests that IS prevalence may be particularly influenced by contaminants/conditions present in wastewater, even compared to highly co-selected AMR genes [33].

3.5. No AMR detectable in groundwater around WWTPs

Whilst high NO_3^- in groundwater near to the WWTP was suggestive of surface-derived contamination (Fig. 3d), AMR revealed a contrasting pattern. Although 16S rRNA was detected in both groundwater samples $2.7 \times 10^1 - 2.0 \times 10^2$ copies/mL, AMR genes MGEs nor taxonomic genes were detected in groundwater (50–120 m depth). Whilst a small sample size ($n = 2$) was utilised, both sampling points were located downgradient from the Santiago Metropolitan Region and nearby to the WWTP, reflecting a high likelihood of urban-derived contamination. Given the proximity to potential contaminants and noting that numerous AMR genes in agricultural soils were detected nearby in the Aconcagua Valley, Valparaíso [71], it is surprising that AMR genes were below detection limits. The absence of detectable AMR genes in groundwater may reflect a combination of factors including dilution, sorption, microbial competition and limited connectivity between surface water and deeper aquifers. It is perhaps most likely that the sampled wells represent relatively evolved groundwater with longer residence times, compared to river water, which may reduce the likelihood of detecting recently introduced microbial markers.

4. Conclusions

Longitudinal trends in Maipo River basin water quality and AMR were investigated. Generally, wastewater and AMR-related indicators increased from Andean headwaters to Santiago. Headwaters of the Mapocho were relatively enriched in Mn, Cu, Zn and *folP* prevalence, whilst Maipo headwaters were higher in SO_4^{2-} and EC. Wastewater tracers (Tryp-like fluorescence, log(16S rRNA), DOC, *crAss64,* PO_4) indicated strong associations with IS*1247* ($p < 0.01$), whilst DOC, PO_4, and log(16S rRNA; $p = 0 - 0.05$) were associated with *sul1*. These observations suggest that wastewater discharge may be associated with increased prevalence of AMR-related markers in downstream river water. However, given the limited sample number and potential co-variation with other environmental factors, the data cannot distinguish between direct selection within wastewater systems and downstream enrichment linked to broader anthropogenic pressures. Without indication of the species diversity of the samples, along with increased statistical robustness, it is not possible to determine whether AMR prevalence was controlled *in-situ* by sublethal selective agents or any other process. There were no detectable AMR genes in groundwater around the WWTP, suggesting that exposure to AMR-related health risks in sampled groundwater was low, noting a small sample number. This study warrants further investigation, including with respect to temporal variation, but nevertheless presents important findings on how treated wastewater affects downstream water quality in populated river basins.

CRediT authorship contribution statement

George J.L. Wilson: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Ismael Casado:** Investigation, Writing – review & editing. **Daren C. Gooddy:** Funding acquisition, Resources, Writing – review & editing. **Linda Daniele:** Funding acquisition, Project administration, Resources, Writing – review & editing. **Laura A. Richards:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

Data availability statement

The datasets generated and/or analysed during the current study are not publicly available for data confidentiality reasons but may be available from the corresponding author on reasonable request.

Ethics approval

The authors declare that this study does not involve animals.

Declaration of generative AI and AI-assisted technologies

The authors declare that they did not use generative AI and/or AI-assisted technologies in the manuscript preparation process.

Funding statement

This work was funded by The University of Manchester's Research Collaboration Fund 2023/24, a Dame Kathleen Ollerenshaw Fellowship to L.A.R., UKRI grant MR/Y016327/1 to L.A.R. et al. (www.aquaroad.org) and a 2023 International Science Partnerships Fund award via University of Manchester to L.A.R.

Declaration of competing interest

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

Acknowledgements

Cristobal Alonso Peralta and Alfonso Mohor Alarcón (formerly Universidad de Chile) assisted with sample collection and field logistics. Resistomap Oy is thanked for analytical support and advice. Abby Ragazzon-Smith, Rosie Byrne, Jordan Gaskell, Derick Muloogi (The University of Manchester), and Peter Williams (British Geological Survey) are acknowledged for technical support. The views expressed here do not necessarily represent those of the institutions, funders, or individuals whose support is acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.emcon.2026.100723.

References

[1] M. Naghavi, S.E. Vollset, K.S. Ikuta, L.R. Swetschinski, A.P. Gray, E.E. Wool, G. Robles Aguilar, T. Mestrovic, G. Smith, C. Han, R.L. Hsu, J. Chalek, D.T. Araki, E. Chung, C. Raggi, A. Gershberg Hayoon, N. Davis Weaver, P.A. Lindstedt, A.E. Smith, U. Altay, N.V. Bhattacharjee, K. Giannakis, F. Fell, B. McManigal, N. Ekapirat, J.A. Mendes, T. Runghien, O. Srimokla, A. Abdelkader, S. Abd-Elsalam, R.G. Aboagye, H. Abolhassani, H. Abualruz, U. Abubakar, H.J. Abukhadijah, S. Aburuz, A. Abu-Zaid, S. Achalapong, I.Y. Addo, V. Adekanmbi, T.E. Adeyeoluwa, Q.E.S. Adnani, L.A. Adzigbli, M.S. Afzal, S. Afzal, A. Agodi, A.J. Ahlstrom, A. Ahmad, S. Ahmad, T. Ahmad, A. Ahmadi, A. Ahmed, H. Ahmed, I. Ahmed, M. Ahmed, S. Ahmed, S.A. Ahmed, M.A. Akkaif, S. Al Awaidy, Y. Al Thaher, S.O. Alalalmeh, M.T. AlBataineh, W.A. Aldhaleei, A.A.S. Al-Gheethi, N.B. Alhaji, A. Ali, L. Ali, S.S. Ali, W. Ali, K. Allel, S. Al-Marwani, A. Alrawashdeh, A. Altaf, A.B. Al-Tammemi, J.A. Al-Tawfiq, K.H. Alzoubi, W.A. Al-Zyoud, B. Amos, J.H. Amuasi, R. Ancuceanu, J.R. Andrews, A. Anil, I.A. Anuoluwa, S. Anvari, A.E. Anyasodor, G.L.C. Apostol, J. Arabloo, M. Arafat, A.Y. Aravkin, D. Areda, A. Aremu, A.A. Artamonov, E.A. Ashley, M.O. Asika, S.S. Athari, M.M.W. Atout, T. Awoke, S. Azadnajafabad, J.M. Azam, S. Aziz, A.Y. Azzam, M. Babaei, F.-X. Babin, M. Badar, A.A. Baig, M. Bajcetic, S. Baker, M. Bardhan, H.J. Barqawi, Z. Basharat, A. Basiru, M. Bastard, S. Basu, N.S. Bayleyegn, M.A. Belete, O.O. Bello, A. Beloukas, J.A. Berkley, A.S. Bhagavathula, S. Bhaskar, S.S. Bhuyan, J.A. Bielicki, N.I. Briko, C.S. Brown, A.J. Browne, D. Buonsenso, Y. Bustanji, C.G. Carvalheiro, C.A. Castañeda-Orjuela, M. Cenderadewi, J. Chadwick, S. Chakraborty, R.M. Chandika, S. Chandy, V. Chansamouth, V.K. Chattu, A.A. Chaudhary, P.R. Ching, H. Chopra, F.R. Chowdhury, D.-T. Chu, M. Chutiyami, N. Cruz-Martins, A.G. Da Silva, O. Dadras, X. Dai, S.D. Darcho, S. Das, F.P. De La Hoz, D.M. Dekker, K. Dhama, D. Diaz, B.F.R. Dickson, S.G. Djorie, M. Dodangeh, S. Dohare, K.G. Dokova, O.P. Doshi, R.K. Dowou, H.L. Dsouza, S.J. Dunachie, A.M. Dziedzic, T. Eckmanns, A. Ed-Dra, A. Eftekharimehrabad, T.C. Ekundayo, I. El Sayed, M. Elhadi, W. El-Huneidi, C. Elias, S.J. Ellis, R. Elsheikh, I. Elsohaby, C. Eltaha, B. Eshrati, M. Eslami, D.W. Eyre, A.O. Fadaka, A.F. Fagbamigbe, A. Fahim, A. Fakhri-Demeshghieh, F.O. Fasina, M.M. Fasina, A. Fatehizadeh, N.A. Feasey, A. Feizkhah, G. Fekadu, F. Fischer, I. Fitriana, K.M. Forrest, C. Fortuna Rodrigues, J.E. Fuller, M.A. Gadanya, M. Gajdács, A.P. Gandhi, E.E. Garcia-Gallo, D.O. Garrett, R.K. Gautam, M.W. Gebregergis, M. Gebrehiwot, T.G. Gebremeskel, C. Geffers, L. Georgalis, R.M. Ghazy, M. Golechha, D. Golinelli, M. Gordon, S. Gulati, R.D. Gupta, S. Gupta, V.K. Gupta, A.D. Habteyohannes, S. Haller, H. Harapan, M.L. Harrison, A.I. Hasaballah, I. Hasan, R.S. Hasan, H. Hasani, A.H. Haselbeck, M.S. Hasnain, I.I. Hassan, S. Hassan, M.S. Hassan Zadeh Tabatabaei, K. Hayat, J. He, O.E. Hegazi, M. Heidari, K. Hezam, R. Holla, M. Holm, H. Hopkins, M.M. Hossain, M. Hosseinzadeh, S. Hostiuc, N.R. Hussein, L.D. Huy, E.D. Ibáñez-Prada, A. Ikiroma, I.M. Ilic, S.M.S. Islam, F. Ismail, N.E. Ismail, C.D. Iwu, C.J. Iwu-Jaja, A. Jafarzadeh, F. Jaiteh, R. Jalilzadeh Yengejeh, R.D.G. Jamora, J. Javidnia, T. Jawaid, A.W.J. Jenney, H.J. Jeon, M. Jokar, N. Jomehzadeh, T. Joo, N. Joseph, Z. Kamal, K.K. Kanmodi, R.S. Kantar, J.A. Kapisi, I.M. Karaye, Y.S. Khader, H. Khajuria, N. Khalid, F. Khamesipour, A. Khan, M.J. Khan, M.T. Khan, V. Khanal, F.F. Khidri, J. Khubchandani, S. Khusuwan, M.S. Kim, A. Kisa, V.A. Korshunov, F. Krapp, R. Krumkamp, M. Kuddus, M. Kulimbet, D. Kumar, E.A.P. Kumaran, A. Kuttikkattu, H.H. Kyu, I. Landires, B.K. Lawal, T.T.T. Le, I.M. Lederer, M. Lee, S.W. Lee, A. Lepape, T.L. Lerango, V.S. Ligade, C. Lim, S.S. Lim, L.W. Limenh, C. Liu, Xiaofeng Liu, Xuefeng Liu, M.J. Loftus, H.I. M Amin, K.L. Maass, S.B. Maharaj, M.A. Mahmoud, P. Maikanti-Charalampous, O.M. Makram, K. Malhotra, A.A. Malik, G.D. Mandilara, F. Marks, B.A. Martinez-Guerra, M. Martorell, H. Masoumi-Asl, A.G. Mathioudakis, J. May, T.A. McHugh, J. Meiring, H.N. Meles, A. Melese, E.B. Melese, G. Minervini, N.S. Mohamed, S. Mohammed, S. Mohan, A.H. Mokdad, L. Monasta, A. Moodi Ghalibaf, C.E. Moore, Y. Moradi, E. Mossialos, V. Mougin, G.D. Mukoro, F. Mulita, B. Muller-Pebody, E. Murillo-Zamora, S. Musa, P. Musicha, L.A. Musila, S. Muthupandian, A.J. Nagarajan, P. Naghavi, F. Nainu, T.S. Nair, H.H.R. Najmuldeen, Z.S. Natto, J. Nauman, B.P. Nayak, G.T. Nchanji, P. Ndishimye, I. Negoi, R.I. Negoi, S.A. Nejadghaderi, Q.P. Nguyen, E.A. Noman, D.C. Nwakanma, S. O'Brien, T.J. Ochoa, I.A. Odetokun, O.A. Ogundijo, T.R. Ojo-Akosile, S.R. Okeke, O.C. Okonji, A.T. Olagunju, A. Olivas-Martinez, A.A. Olorukooba, P. Olwoch, K.I. Onyedibe, E. Ortiz-Brizuela, O. Osuolale, P. Ounchanum, O.T. Oyeyemi, M.P. P A, J.L. Paredes, R.R. Parikh, J. Patel, S. Patil, S. Pawar, A.Y. Peleg, P. Peprah, J. Perdigão, C. Perrone, I.-R. Petcu, K. Phommasone, Z.Z. Piracha, D. Poddighe, A.J. Pollard, R. Poluru, A. Ponce-De-Leon, J. Puvvula, F.N. Qamar, N.H. Qasim, C.D. Rafai, P. Raghav, L. Rahbarnia, F. Rahim, V. Rahimi-Movaghar, M. Rahman, M.A. Rahman, H. Ramadan, S.K. Ramasamy, P.S. Ramesh, P.W. Ramteke, R.K. Rana, U. Rani, M.-M. Rashidi, D. Rathish, S. Rattanavong, S. Rawaf, E.M.M. Redwan, L.F. Reyes, T. Roberts, J.V. Robotham, V.D. Rosenthal, A.G. Ross, N. Roy, K.E. Rudd, C.J. Sabet, B.A. Saddik, M.R. Saeb, U. Saeed, S. Saeedi Moghaddam, W. Saengchan, M. Safaei, A. Saghazadeh, N. Saheb Sharif-Askari, A. Sahebkar, S.S. Sahoo, M. Sahu, M. Saki, N. Salam, Z. Saleem, M.A. Saleh, Y.L. Samodra, A.M. Samy, A. Saravanan, M. Satpathy, A.E. Schumacher, M. Sedighi, S. Seekaew, M. Shafie, P.A. Shah, S. Shahid, M.J. Shahwan, S. Shakoor, N. Shalev, M.A. Shamim, M.A. Shamshirgaran, A. Shamsi, A. Sharifan, R.P. Shastry, M. Shetty, A. Shittu, S. Shrestha, E.E. Siddig, T. Sideroglou, J. Sifuentes-Osornio, L.M.L.R. Silva, E.A.F. Simões, A.J.H. Simpson, A. Singh, S. Singh, R. Sinto, S.S.M. Soliman, S. Soraneh, N. Stoesser, T.Z. Stoeva, C.K. Swain, L. Szarpak, S.S. T Y, S. Tabatabai, C. Tabche, Z.M.-A. Taha, K.-K. Tan, N. Tasak, N.Y. Tat, A. Thaiprakong, P. Thangaraju, C.C. Tigoi, K. Tiwari, M.R. Tovani-Palone, T.H. Tran, M. Tumurkhuu, P. Turner, A.J. Udoakang, A. Udoh, N. Ullah, S. Ullah, A.G. Vaithinathan, M. Valenti, T. Vos, H.T.L. Vu, Y. Waheed, A.S. Walker, J.L. Walson, T. Wangrangsimakul, K.G. Weerakoon, H.F.L. Wertheim, P.C.M. Williams, A.A. Wolde, T.M. Wozniak, F. Wu, Z. Wu, M.K.K. Yadav, S. Yaghoubi, Z.S. Yahaya, A. Yarahmadi, S. Yezli, Y.E. Yismaw, D.K. Yon, C.-W. Yuan, H. Yusuf, F. Zakham, G. Zamagni, H. Zhang, Z.-J. Zhang, M. Zielińska, A. Zumla, S.H.H. Zyoud, S.H. Zyoud, S.I. Hay, A. Stergachis, B. Sartorius, B.S. Cooper, C. Dolecek, C.J.L. Murray, Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050, Lancet 404 (2024) 1199–1226, https://doi.org/10.1016/S0140-6736(24)01867-1.

[2] P. Rattanaumpawan, A. Boonyasiri, S. Vong, V. Thamlikitkul, Systematic review of electronic surveillance of infectious diseases with emphasis on antimicrobial resistance surveillance in resource-limited settings, Am. J. Infect. Control 46 (2018) 139–146, https://doi.org/10.1016/j.ajic.2017.08.006.

[3] WHO, in: WHO Bacterial Priority Pathogens List 2024: Bacterial Pathogens of Public Health Importance, to Guide Research, Development, and Strategies to Prevent and Control Antimicrobial Resistance, first ed., World Health Organization, Geneva, 2024.

[4] C. Michaelis, E. Grohmann, Horizontal gene transfer of antibiotic resistance genes in biofilms, Antibiotics 12 (2023) 328, https://doi.org/10.3390/antibiotics12020328.

[5] D.G.J. Larsson, A. Andremont, J. Bengtsson-Palme, K.K. Brandt, A.M. De Roda Husman, P. Fagerstedt, J. Fick, C.-F. Flach, W.H. Gaze, M. Kuroda, K. Kvint, R. Laxminarayan, C.M. Manaia, K.M. Nielsen, L. Plant, M.-C. Ploy, C. Segovia, P. Simonet, K. Smalla, J. Snape, E. Topp, A.J. Van Hengel, D.W. Verner-Jeffreys, M.P.J. Virta, E.M. Wellington, A.-S. Wernersson, Critical knowledge gaps and research needs related to the environmental dimensions of antibiotic resistance, Environ. Int. 117 (2018) 132–138, https://doi.org/10.1016/j.envint.2018.04.041.

[6] D.G.J. Larsson, C.F. Flach, Antibiotic resistance in the environment, Nat. Rev. Microbiol. 20 (2021) 257–269, https://doi.org/10.1038/s41579-021-00649-x, 2021 205.

[7] Z. Yu, D.A. Gray, J. Fick, N. Waters, R. Lindberg, R. Grabic, M. Tysklind, M.D. Ekwanzala, H.-M. Martiny, C.-F. Flach, F.M. Aarestrup, D.G.J. Larsson, Antibiotic resistance selection and deselection in municipal wastewater from 47 countries, Nat. Commun. 16 (2025) 9698, https://doi.org/10.1038/s41467-025-65670-7.

[8] D.I. Andersson, D. Hughes, Microbiological effects of sublethal levels of antibiotics, Nat. Rev. Microbiol. 12 (2014) 465–478, https://doi.org/10.1038/nrmicro3270.

[9] C.L. Brown, A. Maile-Moskowitz, A.J. Lopatkin, K. Xia, L.K. Logan, B.C. Davis, L. Zhang, P.J. Vikesland, A. Pruden, Selection and horizontal gene transfer underlie microdiversity-level heterogeneity in resistance gene fate during wastewater treatment, Nat. Commun. 15 (2024) 5412, https://doi.org/10.1038/s41467-024-49742-8.

[10] A. Pruden, M. Arabi, H.N. Storteboom, Correlation between upstream human activities and riverine antibiotic resistance genes, Environ. Sci. Technol. 46 (2012) 11541–11549, https://doi.org/10.1021/es302657r.

[11] G.J. Wilson, M. Perez-Zabaleta, I. Owusu-Agyeman, A. Kumar, A. Ghosh, D.A. Polya, D.C. Gooddy, Z. Cetecioglu, L.A. Richards, Discovery of sulfonamide resistance genes in deep groundwater below Patna, India, Environ. Pollut. (2024) 124205, https://doi.org/10.1016/j.envpol.2024.124205.

[12] Z. Jiang, J. Zeng, X. Wang, H. Yu, L. Yue, C. Wang, F. Chen, Z. Wang, Biodegradable microplastics and dissemination of antibiotic resistance genes: an undeniable risk associated with plastic additives, Environ. Pollut. 372 (2025) 125952, https://doi.org/10.1016/j.envpol.2025.125952.

[13] C. Pal, K. Asiani, S. Arya, C. Rensing, D.J. Stekel, D.G.J. Larsson, J.L. Hobman, Metal resistance and its Association with antibiotic resistance, in: Advances in Microbial Physiology, Elsevier, 2017, pp. 261–313, https://doi.org/10.1016/bs.ampbs.2017.02.001.

[14] X. Tong, Z. Xiang, S.G. Goh, L. You, S. Mohapatra, G. Ong, W.C. Khor, K.T. Aung, K.Y.-H. Gin, A hybrid modeling framework for predicting spatiotemporal dynamics of antimicrobial resistance in coastal waters, Environ. Sci. Technol. (2025), https://doi.org/10.1021/acs.est.5c01927.

[15] A. Wales, R. Davies, Co-Selection of resistance to antibiotics, biocides and heavy metals, and its relevance to foodborne pathogens, Antibiotics 4 (2015) 567–604, https://doi.org/10.3390/antibiotics4040567.

[16] X. Yu, Z.-C. Zhou, X. Shuai, Z. Lin, Z. Liu, J. Zhou, Y. Lin, G. Zeng, Z. Ge, H. Chen, Microplastics exacerbate co-occurrence and horizontal transfer of antibiotic resistance genes, J. Hazard Mater. 451 (2023) 131130, https://doi.org/10.1016/j.jhazmat.2023.131130.

[17] K. Blau, A. Bettermann, S. Jechalke, E. Fornefeld, Y. Vanrobaeys, T. Stalder, E.M. Top, K. Smalla, The transferable resistome of produce, mBio 9 (2018) 10–1128.

[18] T. Lima, S. Domingues, G.J. Da Silva, Manure as a potential hotspot for antibiotic resistance dissemination by horizontal gene transfer events, Vet. Sci. 7

(2020) 110, https://doi.org/10.3390/vetsci7030110.
[19] D.R. MacFadden, S.F. McGough, D. Fisman, M. Santillana, J.S. Brownstein, Antibiotic resistance increases with local temperature, Nat. Clim. Change 8 (2018) 510–514, https://doi.org/10.1038/s41558-018-0161-6.
[20] K. Poole, Stress responses as determinants of antimicrobial resistance in Gram-negative bacteria, Trends Microbiol. 20 (2012) 227–234.
[21] J. Zhang, J. Chen, C. Wang, P. Wang, B. Feng, H. Gao, D. Chen, Nitrate input enriched the antibiotic resistance genes in lake sediments by shaping co-host community and promoting horizontal gene transfer, J. Hazard Mater. 497 (2025) 139580, https://doi.org/10.1016/j.jhazmat.2025.139580.
[22] H. Baba, M. Nishiyama, T. Watanabe, H. Kanamori, Review of antimicrobial resistance in wastewater in Japan: current challenges and future perspectives, Antibiotics 11 (2022) 1–19, https://doi.org/10.3390/antibiotics11070849.
[23] I. Bueno, C. Verdugo, O. Jimenez-Lopez, P.P. Alvarez, G. Gonzalez-Rocha, C.A. Lima, D.A. Travis, B. Wass, Q. Zhang, S. Ishii, R.S. Singer, Role of wastewater treatment plants on environmental abundance of Antimicrobial Resistance Genes in Chilean rivers, Int. J. Hyg Environ. Health 223 (2020) 56–64, https://doi.org/10.1016/j.ijheh.2019.10.006.
[24] B.C. Davis, M.V. Riquelme, G. Ramirez-Toro, C. Bandaragoda, E. Garner, W.J. Rhoads, P. Vikesland, A. Pruden, Demonstrating an integrated antibiotic resistance gene surveillance approach in Puerto Rican watersheds post-hurricane maria, Environ. Sci. Technol. 54 (2020) 15108–15119, https://doi.org/10.1021/acs.est.0c05567.
[25] M.E. Knight, M. Wade, G. Webster, D.A. Pass, W. Perry, P. Kille, A. Singer, D.L. Jones, Wastewater-based analysis of antimicrobial resistance at UK airports: evaluating the potential opportunities and challenges, Environ. Int. (2025) 109260, https://doi.org/10.1016/j.envint.2025.109260.
[26] M. Munir, K. Wong, I. Xagoraraki, Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan, Water Res. 45 (2011) 681–693, https://doi.org/10.1016/j.watres.2010.08.033.
[27] Y. Tang, Z. Liang, G. Li, H. Zhao, T. An, Metagenomic profiles and health risks of pathogens and antibiotic resistance genes in various industrial wastewaters and the associated receiving surface water, Chemosphere 283 (2021) 131224, https://doi.org/10.1016/j.chemosphere.2021.131224.
[28] P. Yu, X. Guo, W. Wang, L. Wang, H. Zhang, L. Deng, H. Yang, T. He, P. Wu, Y. Zhang, Distribution and driving mechanisms of antibiotic resistance genes in urbanized watersheds, Sci. Total Environ. 954 (2024) 176387, https://doi.org/10.1016/j.scitotenv.2024.176387.
[29] J. Bengtsson-Palme, R. Hammarén, C. Pal, M. Östman, B. Björlenius, C.-F. Flach, J. Fick, E. Kristiansson, M. Tysklind, D.G.J. Larsson, Elucidating selection processes for antibiotic resistance in sewage treatment plants using metagenomics, Sci. Total Environ. 572 (2016) 697–712, https://doi.org/10.1016/j.scitotenv.2016.06.228.
[30] D.S. Read, H.S. Gweon, M.J. Bowes, M.F. Anjum, D.W. Crook, K.K. Chau, L.P. Shaw, A. Hubbard, M. AbuOun, H.J. Tipper, S.J. Hoosdally, M.J. Bailey, A.S. Walker, N. Stoesser, Dissemination and persistence of antimicrobial resistance (AMR) along the wastewater-river continuum, Water Res. 264 (2024) 122204, https://doi.org/10.1016/j.watres.2024.122204.
[31] Y. Yang, B. Li, S. Zou, H.H.P. Fang, T. Zhang, Fate of antibiotic resistance genes in sewage treatment plant revealed by metagenomic approach, Water Res. 62 (2014) 97–106, https://doi.org/10.1016/j.watres.2014.05.019.
[32] M.M. Rahman, M.R. Alam, N. Siboni, M.A. Halim, K.R. Mahbub, Z. Rogers, J.R. Seymour, The fate of antibiotic resistance genes in tertiary wastewater treatment works and their correlation with other contaminants, Clean. Water 6 (2026) 100223, https://doi.org/10.1016/j.clwat.2026.100223.
[33] F. Berglund, S. Ebmeyer, E. Kristiansson, D.G.J. Larsson, Evidence for wastewaters as environments where mobile antibiotic resistance genes emerge, Commun. Biol. 6 (2023) 321, https://doi.org/10.1038/s42003-023-04676-7.
[34] A. Baker, Fluorescence properties of some farm wastes: implications for water quality monitoring, Water Res. 36 (2002) 189–195, https://doi.org/10.1016/S0043-1354(01)00210-X.
[35] C. García-Aljaro, E. Ballesté, M. Muniesa, J. Jofre, Determination of crAssphage in water samples and applicability for tracking human faecal pollution, Microb. Biotechnol. 10 (2017) 1775–1780, https://doi.org/10.1111/1751-7915.12841.
[36] A. Karkman, K. Pärnänen, D.G.J. Larsson, Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments, Nat. Commun. 10 (2019) 80, https://doi.org/10.1038/s41467-018-07992-3.
[37] S. Morales-Cortés, L. Sala-Comorera, C. Gómez-Gómez, M. Muniesa, C. García-Aljaro, CrAss-like phages are suitable indicators of antibiotic resistance genes found in abundance in fecally polluted samples, Environ. Pollut. 359 (2024) 124713, https://doi.org/10.1016/j.envpol.2024.124713.
[38] I. Vera-Puerto, H. Valdés, M. Bueno, C. Correa, J. Olave, M. Carrasco-Benavides, F. Schiappacasse, C.A. Arias, Reclamation of treated wastewater for irrigation in Chile: perspectives of the Current State and challenges, Water 14 (2022) 627, https://doi.org/10.3390/w14040627.
[39] S.-Y.-D. Zhou, M.-Y. Wei, M. Giles, R. Neilson, F. Zheng, Q. Zhang, Y.-G. Zhu, X.-R. Yang, Prevalence of antibiotic resistome in ready-to-eat salad, Front. Public Health 8 (2020) 92, https://doi.org/10.3389/fpubh.2020.00092.
[40] D.C. Domínguez, L.M. Chacón, D. Wallace, Anthropogenic activities and the problem of antibiotic resistance in Latin America: a water issue, Water 13 (2021) 2693, https://doi.org/10.3390/w13192693.
[41] MINSAL, Plan nacional contra la Resistencia a los antimicrobianos, Ministerio de Salud de Chile (2017).
[42] A.I. Moreno-Switt, D. Rivera, M.L. Caipo, D.C. Nowell, A.D. Adell, Antimicrobial resistance in water in Latin America and the Caribbean: available research and gaps, Front. Vet. Sci. 7 (2020).
[43] INE, Censo 2024, 2024.
[44] SUEZ, n.d. La Farfana Wastewater Treatment Plant.
[45] V. Irarrazaval, R.H. Sillitoe, A.J. Wilson, J.C. Toro, W. Robles, G.D. Lyall, Discovery History of a Giant, high-grade, Hypogene Porphyry copper-molybdenum Deposit at Los Sulfatos, Los Bronces-Río Blanco District, Central Chile, 2010.
[46] J. Pizarro, P.M. Vergara, J.A. Rodríguez, A.M. Valenzuela, Heavy metals in northern Chilean rivers: spatial variation and temporal trends, J. Hazard Mater. 181 (2010) 747–754, https://doi.org/10.1016/j.jhazmat.2010.05.076.
[47] R. Segura, V. Arancibia, M.C. Zúñiga, P. Pastén, Distribution of copper, zinc, lead and cadmium concentrations in stream sediments from the Mapocho River in Santiago, Chile, J. Geochem. Explor. 91 (2006) 71–80, https://doi.org/10.1016/j.gexplo.2006.03.003.
[48] B. Lehner, G. Grill, Global river hydrography and network routing: baseline data and new approaches to study the world's large river systems, Hydrol. Process. 27 (2013) 2171–2186, https://doi.org/10.1002/hyp.9740.
[49] NASA Shuttle Radar Topography Mission (SRTM), Shuttle Radar Topography Mission (SRTM) global. https://doi.org/10.5069/G9445JDF, 2013.
[50] OpenStreetMap contributors, Openstreetmap, 2025.
[51] Resistomap, Resistomap, 2025. URL resistomap.cpm. (Accessed 6 June 2025).
[52] W.I. Muziasari, K. Pärnänen, T.A. Johnson, C. Lyra, A. Karkman, R.D. Stedtfeld, M. Tamminen, J.M. Tiedje, M. Virta, Aquaculture changes the profile of antibiotic resistance and mobile genetic element associated genes in Baltic Sea sediments, FEMS Microbiol. Ecol. 92 (2016) fiw052, https://doi.org/10.1093/femsec/fiw052.
[53] R.D. Stedtfeld, X. Guo, T.M. Stedtfeld, H. Sheng, M.R. Williams, K. Hauschild, S. Gunturu, L. Tift, F. Wang, A. Howe, B. Chai, D. Yin, J.R. Cole, J.M. Tiedje, S.A. Hashsham, Primer set 2.0 for highly parallel qPCR array targeting antibiotic resistance genes and mobile genetic elements, FEMS Microbiol. Ecol. 94 (2018) fiy130, https://doi.org/10.1093/femsec/fiy130.
[54] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative CT method, Nat. Protoc. 3 (2008) 1101–1108, https://doi.org/10.1038/nprot.2008.73.
[55] J.S.T. Ward, D.J. Lapworth, D.S. Read, S. Pedley, S.T. Banda, M. Monjerezi, G. Gwengweya, A.M. Macdonald, Tryptophan-like fluorescence as a high-level screening tool for detecting microbial contamination in drinking water, Sci. Total Environ. 750 (2021) 141284, https://doi.org/10.1016/j.scitotenv.2020.141284.
[56] D.J. Lapworth, D.G. Kinniburgh, An R script for visualising and analysing fluorescence excitation–emission matrices (EEMs), Comput. Geosci. 35 (2009) 2160–2163, https://doi.org/10.1016/j.cageo.2008.10.013.
[57] J.R. Lakowicz, Topics in Fluorescence Spectroscopy: Volume 4: Probe Design and Chemical Sensing, Springer Science & Business Media, 1994.
[58] L.A. Richards, D.J. Lapworth, D. Magnone, D.C. Gooddy, L. Chambers, P.J. Williams, B.E. van Dongen, D.A. Polya, Dissolved organic matter tracers reveal contrasting characteristics across high arsenic aquifers in Cambodia: a fluorescence spectroscopy study, Geosci. Front. 10 (2019) 1653–1667, https://doi.org/10.1016/j.gsf.2019.04.009.
[59] R Core Team, R: a Language and Environment for Statistical Computing, 2023.
[60] Z. Gu, Complex heatmap visualization, iMeta 1 (2022) e43, https://doi.org/10.1002/imt2.43.
[61] Z. Gu, R. Eils, M. Schlesner, Complex heatmaps reveal patterns and correlations in multidimensional genomic data, Bioinformatics 32 (2016) 2847–2849, https://doi.org/10.1093/bioinformatics/btw313.
[62] M.D. Peña-Guerrero, A. Nauditt, C. Muñoz-Robles, L. Ribbe, F. Meza, Drought impacts on water quality and potential implications for agricultural production in the Maipo River Basin, Central Chile, Hydrol. Sci. J. 65 (2020) 1005–1021, https://doi.org/10.1080/02626667.2020.1711911.
[63] B. Fernández, J. Gironás, Water Resources of Chile, Springer, 2021.
[64] G.P. Pincetti-Zúniga, L.A. Richards, L. Daniele, A.J. Boyce, D.A. Polya, Hydrochemical characterization, spatial distribution, and geochemical controls on arsenic and boron in waters from arid Arica and Parinacota, northern Chile, Sci. Total Environ. 806 (2022) 150206, https://doi.org/10.1016/j.scitotenv.2021.150206.
[65] T. Andersson, A.D. Adell, A.I. Moreno-Switt, P. Spégel, C. Turner, S. Overballe-Petersen, K. Fuursted, R. Lood, Biogeographical variation in antimicrobial resistance in rivers is influenced by agriculture and is spread through bacteriophages, Environ. Microbiol. 24 (2022) 4869–4884, https://doi.org/10.1111/1462-2920.16122.
[66] M.K. Schussman, S. Feng, A. Schmoldt, M.J. Bootsma, K. Dennis, K.H. Janssen, S.L. McLellan, Environmental reservoirs account for high levels of carbapenem resistance genes in wastewater, Microbiol. Spectr. 14 (2026), https://doi.org/10.1128/spectrum.01737-25 e01737–25.
[67] N.K. Pandey, M. Simon, R.K. Vishwakarma, S. Sen, H. Joshi, S. Yadav, J. Mateo-Sagasta, M. Jampani, A. Sikka, S. Hazra, Exploring microbial diversity, antibiotic resistance, and their environmental drivers in urban and peri-urban riverbed sediments of sub-tropical river basins, Environ. Res. 288 (2026)

123174, https://doi.org/10.1016/j.envres.2025.123174.
[68] P. Vats, U.J. Kaur, P. Rishi, Heavy metal-induced selection and proliferation of antibiotic resistance: a review, J. Appl. Microbiol. 132 (2022) 4058–4076, https://doi.org/10.1111/jam.15492.
[69] N. Wu, S. Xie, M. Zeng, X. Xu, Y. Li, X. Liu, X. Wang, Impacts of pile temperature on antibiotic resistance, metal resistance and microbial community during swine manure composting, Sci. Total Environ. 744 (2020) 140920, https://doi.org/10.1016/j.scitotenv.2020.140920.
[70] O. Sköld, Sulfonamide resistance: mechanisms and trends. Drug resist. Updat. Rev. Comment. Antimicrob, Anticancer Chemother 3 (2000) 155–160, https://doi.org/10.1054/DRUP.2000.0146.
[71] M. Fresno, L. Pavez, Y. Poblete, A. Cortez, T. Del Pozo, Unveiling antimicrobial resistance in Chilean fertilized soils: a one Health perspective on environmental AMR surveillance, Front. Microbiol. 14 (2023) 1239761, https://doi.org/10.3389/fmicb.2023.1239761.