From remote to urbanized: Dispersal of antibiotic-resistant bacteria under the aspect of anthropogenic influence

Daniel Gattinger [a],[*], Valentin Schlenz [a], Tobias Weil [b], Birgit Sattler [a],[c]

[a] Institute of Ecology, University of Innsbruck, Innsbruck, Austria
[b] Research and Innovation Centre, Fondazione Edmund Mach, All'adige, Italy
[c] Austrian Polar Research Institute, Vienna, Austria

HIGHLIGHTS

- Decline in antibiotic efficacy is not limited to clinical settings but is also observed in environmental samples
- Over 50% of isolated microorganisms exhibit multidrug resistance (MDR), indicating limited antibiotic effectiveness
- The decreasing effectiveness of antibiotics, including that of reserve antibiotics, has raised concerns
- Innovative approach assesses anthropogenic impact using Corine Land Cover data and heatmaps from sports activity trackers
- Clear link between increased human influence and higher AR levels in glacier, snow, and lake samples

GRAPHICAL ABSTRACT
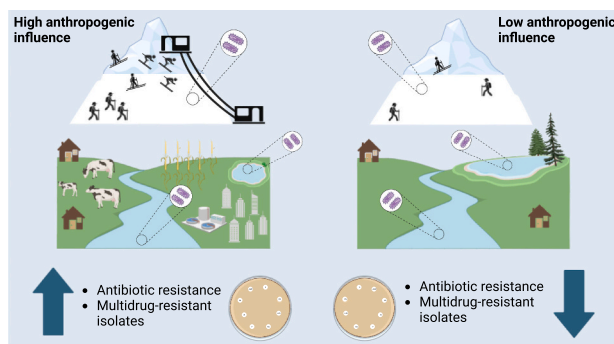
ARTICLE INFO

Editor: Abasiofiok Mark Ibekwe

ABSTRACT

Antibiotic resistance is a growing global concern, but our understanding of the spread of resistant bacteria in remote regions remains limited. While some level of intrinsic resistance likely contributes to reduced susceptibility to antimicrobials in the environment, it is evident that human actions, particularly the (mis)use of antibiotics, play a significant role in shaping the environmental resistome, even in seemingly distant habitats like glacier ice sheets.

Our research aims to bridge this knowledge gap by investigating the direct influence of human activities on the presence of antibiotic-resistant bacteria in various habitats. To achieve a comprehensive assessment of anthropogenic impact across diverse and seemingly isolated sampling sites, we developed an innovative approach utilizing Corine Land Cover data and heatmaps generated from sports activity trackers. This method allowed us to make meaningful comparisons across relatively pristine environments.

Our findings indicate a noteworthy increase in culturable antibiotic-resistant bacteria with heightened human influence, as evidenced by our analysis of glacier, snow, and lake water samples. Notably, the most significant concentrations of antibiotic-resistant and multidrug-resistant microorganisms were discovered in two highly impacted sampling locations, namely the Tux Glacier and Gas Station Ellmau.

* Corresponding author.
  E-mail address: daniel.gattinger@student.uibk.ac.at (D. Gattinger).

1. Introduction

The discovery of Penicillin in the mid-20th century and the following 'golden era' with the discovery of many new classes of antibiotics revolutionized modern medicine. However, the extensive human use of these drugs quickly raised a new challenge in the form of antimicrobial resistance (AMR). Almost 100 years later, bacteria seem to be outperforming human capabilities to find and produce new effective antibiotics. As of 2023, the Comprehensive Antibiotic Resistance Database (CARD) lists over 300.000 alleles that represent known unique variants of antibiotic resistance genes (ARGs) (Alcock et al., 2023) and while the number of AMR continues to rise, the discovery of new effective substances to inhibit bacterial growth has declined drastically (Ventola, 2015) thus posing a worldwide challenge that urgently needs to be tackled (WHO, 2019). Not only does AMR pose a major threat to public health, but it also has economic implications. According to The Review on Antimicrobial Resistance (2016), antibiotic resistance (AR) could cost the global economy up to $100 trillion until 2050 if no action is taken.

Beyond clinical settings, AMR has emerged as a serious environmental issue. There is increasing evidence that AR is not only limited to populated areas but also is of concern for natural environments including the cryosphere (Gattinger et al., 2023).

The cryosphere encompasses a variety of habitats such as glaciers, seasonal snow cover, permafrost, sea ice, and others that were once thought to be void of life. Nowadays, it is well-known that the cryosphere harbors a variety of life which includes bacteria, archaea, viruses, and fungi that have adapted to cold and nutrient-depleted environments (e.g. Jungblut et al., 2022) Moreover, these cold habitats can contain significant numbers of antibiotic-resistant bacteria (Hernández and González-Acuña, 2016; McCann et al., 2019; Ushida et al., 2010; Segawa et al., 2013). Yet, despite these sensible habitats being highly interconnected with other ecosystems, AMR research in the cryosphere is still in its early days and mainly focused on the detection of ARGs.

It is generally agreed that animal migration, global wind and water systems, and human travel activities are potentially contributing to the worldwide spread of AMR (Allen et al., 2010), however, transport mechanisms to remote areas are not yet fully understood.

Antibiotic resistance has mainly originated from the additional selective pressure provoked by extensive human use of antibiotics. Here, a variety of factors like overuse and misuse of antibiotics, inadequate sanitation, and use of antibiotics in agri- and aquaculture have contributed massively to the current state of AMR.

Therefore, it is essential to consider the relevance of anthropogenic influence in the context of AMR within cryospheric habitats. Despite the traditional perception of these habitats as pristine and unaffected by human activities, increasing evidence suggests otherwise. Anthropogenic activities play a crucial role in introducing antimicrobial resistance genes (ARGs) into these environments through various entry pathways that are closely connected to humans like plastic pollutants that act as carriers for ARGs, facilitating their transport into remote glacial environments (Laganà et al., 2019). Similarly, the migration of animals can introduce resistant bacteria into these habitats, contributing to the dissemination of AMR (Laborda et al., 2022). Human activities such as tourism and research expeditions in polar regions can also cause spreading of AMR (Depta and Niedźwiedzka-Rystwej, 2023). Finally, recent research suggests that AMR may be disseminated through atmospheric processes, particularly in mountainous regions, highlighting the complexity of the pathways involved (Rossi et al., 2023).

Despite these entry pathways, it is worth noting that AMR can also emerge within cryospheric habitats independent of direct human influence. Native bacterial populations inhabiting these environments can exhibit intrinsic resistance to antimicrobials, contributing to the overall resistome (Pawlowski et al., 2016). This intrinsic resistance underscores the need for comprehensive research to elucidate the dynamics of AMR in cryospheric habitats, considering both anthropogenic and natural factors.

With this study, we aimed to achieve a better understanding of the human impact on AMR in different environmental habitats including cryospheric and freshwater samples. To this end, we analyzed available data that are usually considered sources for AR such as direct human presence and land cover data associated with hotspots for AR.

Our samples included a) glacial snow and ice, b) seasonal snow in areas with different levels of human impact as well as c) river systems (from melting waters to larger rivers), and d) different types of lakes (high altitude to swimming lakes).

The spanning from remote to urbanized areas allowed us to compare the prevalence of AMR in culturable bacteria at different levels of anthropogenic influence (low, medium, and high).

By analyzing the data collected from various environmental and cryospheric sources, the work presented here paves the way for a better understanding of the influence of human activities on the spread of AR in our environment.

2. Material and methods

2.1. Sampling sites

Snow, ice, and water were sampled during several field campaigns between November 2018 and July 2020 (Fig. 1). Sampling sites were chosen based on three different levels of anthropogenic influence (low, medium, high), which was determined by several factors such as direct human activity (glaciers), disposal/catchment areas, and land use data (lakes, rivers, snow).

Glacier samples consisted of snow as well as ice samples and were taken from the surface of the glacial ice sheets. The Tux Glacier was a unique sampling site in this study due to sampling in a walkable crevasse (Nature Ice Palace [NIP]) resembling a cave being available for tourism. Samples were mostly taken from a variety of locations within the ice cave with 50,000 visitors/year, (pers. comm. Roman Erler).

2.2. Evaluating anthropogenic influence

It is impossible to determine the absolute human impact with precision because there is no single reliable parameter that comprehensively accounts for all the diverse factors that contribute to it. However, some indicators can be used to differentiate between different levels of anthropogenic influence. These include among others human presence, population density, land use, sewage, and disposal area.

To compare anthropogenic influence between sampling sites, catchment areas for snow and lakes were delineated using QGIS (QGIS Version 3.16) with the GRASS and PCRraster add-on using a digital elevation model of Tyrol with a resolution of 5 m (Land Tirol - data.tirol. gv.at, 2023). Disposal areas derived from Tiris Maps provided by the Amt der Tiroler Landesregierung (2023) were used as the relevant area to evaluate human impact on flowing waters.

Within catchment and disposal areas, the percentage of anthropogenically used land was calculated based on the freely available CORINE Land Cover (CLC) data provided by the European Union (2018). For that purpose, levels of the CLC were reclassified and aggregated to fit into 5 major categories: artificial surfaces, agricultural areas, forests and semi-natural areas, wetlands, and water. Snow, lake, and water sampling sites have then been categorized into low (< 33 % artificial/agricultural areas), medium (33–66 % artificial/agricultural areas), and high (> 66 % artificial/agricultural areas).

For snow and ice from glacial samples, the main indicator of human impact was direct human presence. To compare relevant areas, heat maps (Strava, 2023) were used. These color-coded heatmaps (see Supplementary 1) represent the relative abundance of human activity in specific regions with brighter colors usually indicating higher activity. Using this information we calculated the percentage of the area of interest (glacier surface) subjected to varying levels of direct human

impact. Color channels were therefore split into four categories depicting no, low, medium, or high human presence at a specific pixel.

2.3. Sampling

Snow and superficial ice were scratched off with a cleaned stainless-steel shovel whereas deeper layers of ice were sampled with a Kovacs Ice Corer (Mark III, ø 7,25 cm). The respective original sample was used to clean all sampling devices. Snow and ice samples were sampled in Whirl-Packs[R] and stored at −20 °C until further use.

Water was collected into 250 ml sterile screw-cap bottles, transported to the home institute, and stored at 4 °C before proceeding with cultivation experiments.

2.4. Isolation of pure cultures

To isolate pure cultures 1 ml of each snow, ice, and water sample was plated on Reasoner's 2 A agar (R2A, Merck) and incubated at 4 °C and 20 °C respectively until visible growth was observed. The choice of incubation temperature was a compromise in terms of the tolerability of cold-loving bacteria and temperatures as close as possible to the official recommendation by the EUCAST. All samples were treated the same way except for an initial melting step at 4 °C for snow and ice samples kept at −20 °C after sampling. R2A agar was selected due to its oligotrophic properties which fit the preference of cryospheric organisms.

Distinct colonies were described for morphology, color, and optical growth before the transfer into 7 ml of liquid media (lysogeny broth, LB) with a sterile loop. Seven days of incubation in LB media at the respective temperatures allowed for sufficient biomass growth to perform further tests. The purity of all isolates was then confirmed by streaking. LB was the media of choice due to its suitability for subsequent agar disk diffusion tests.

2.5. Agar disk diffusion test

To perform the agar disk diffusion test standardized by EUCAST (eucast.org, 2021), some modifications were necessary for psychrophilic, slow-growing organisms (Gattinger et al., 2023). In brief, these changes involved the reduction of the incubation temperature from 37 °C to 20 and 4 °C respectively as well as the extension of the incubation time to up to two weeks for slow-growing bacteria. The lack of breakpoints for non-pathogenic bacteria was overcome by calculating all available diameter breakpoints from the official guidelines (eucast.org, 2023, see Supplementary 1). For novobiocin, no diameters are listed in the EUCAST breakpoint table. Therefore, thresholds were applied based on existing literature (Harrington and Gaydos, 1984).

Besides these adjustments, we adhered to the official guidelines for the agar disk diffusion method.

Following the EUCAST reading guide, bacteria with a zone of inhibition smaller than the defined breakpoint are classified as resistant. Isolates that showed an inhibition zone size equaled or exceeded the calculated thresholds were considered sensitive to the antibiotic.

2.6. Antibiotics

A total of 8 antibiotics were used to test the susceptibility of isolated bacteria. These covered a wide range in mechanisms of action (including but not limited to inhibition of translation, transcription, and cell wall biosynthesis), antibiotic synthesis (natural, semi-synthetic, synthetic), the latest WHO AWaRe classification (access, watch, reserve), and suitability for the agar disk diffusion test (Table 1). Strains were classified as multidrug-resistant (MDR) when they were resistant to at least three different antimicrobials.
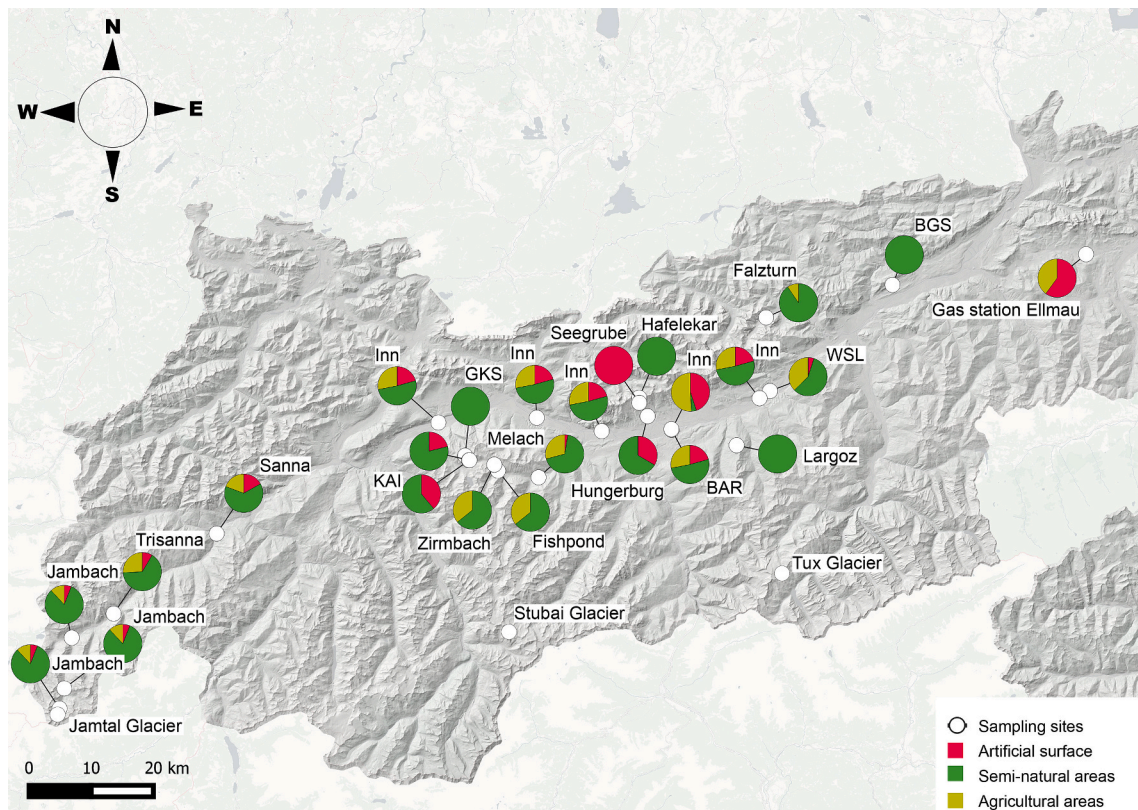

Fig. 1. Sampling sites throughout the Tyrolean alpine space including an overview of the properties of the surrounding land cover from CLC. Glacier samples were not investigated for land cover but rather heatmaps from tracking services.

2.7. DNA extraction

The DNA of all isolated and purified cultures was extracted using the DNeasy Blood & Tissue kit (Qiagen). As starting material for the extraction, bacterial isolates were inoculated in 5 ml of LB media for 7–14 days. After centrifugation, all further steps were performed following the protocol for bacterial isolates provided by the manufacturer. DNA quantity and quality were evaluated with a nanodrop spectrophotometer.

2.8. PCR and Sanger sequencing

PCR amplification was performed by targeting the hypervariable regions V1 to V9 of the 16S rRNA gene with the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGT-TACGACTT-3'). PCR reactions were carried out using 2× PCR Master Mix (Thermo Fisher) and 50 ng template DNA. The cycling conditions consisted of an initial denaturation at 95 °C for 2 min, followed by 30 cycles at 95 °C for 30 s, annealing at 60 °C for 30 s, elongation at 72 °C for 1 min, and a final elongation step at 72 °C for 7 min. PCR products were checked by gel electrophoresis and purified using the ExoSAP-IT™ reagent (Applied Biosystems) according to the manufacturer's instructions. The purified PCR products were sequenced at the Fondazione Edmund Mach on a 3730xl Genetic Analyzer (Applied Biosystems).

2.9. Bioinformatics and statistics

Data generated through sequencing underwent initial processing using the sangeranalyseR package (Chao et al., 2021) in R (version 4.2.2) for quality trimming (QS 30). This allowed the generation of Sanger reports and fasta files which were then used to perform a BLAST search against the NCBI database.

To calculate the glacier area with human activity from heatmaps, R together with the 'countcolors' package (Hooper et al., 2020) was used.

For the statistical analysis, R (version 4.2.2) was utilized with the packages 'dplyr', 'ggpubr', and 'ggplot2'. After testing for assumptions, ANOVA was employed to assess the impact of the anthropogenic influence on AR in environmental and cryospheric sampling sites. Subsequently, a Tukey post hoc test was conducted to identify significant pairwise differences between the groups.

In cases where one or more assumptions of the ANOVA were not met and data transformation did not suffice, a Kruskal-Wallis test followed by a Pairwise Wilcoxon test was performed.

3. Results

3.1. Human activity heatmaps

Tracking data from one of the main providers (Strava), was used to estimate the relative direct human presence on each glacier of interest. The highest anthropogenic activity was observed in the glacial skiing areas on the Tux and Stubai Glacier. Additionally, samples from Tux Glacier were taken within the NIP. Data collected revealed that the human impact on the Jamtal Glacier is low, on the Stubai Glacier

medium, and high on the Tux Glacier (Table 2).

3.2. Corine land use data

The CLC data of the respective catchment areas of snow and lake samples as well as of the disposal area of flowing waters revealed that one lake (BAR) and two snow sampling sites (Seegrube, Ellmau) are under high anthropogenic pressure. On the contrary, no flowing water was found to be highly influenced by humans, and for three snow samples (BGS, Hafelakar, Largoz) and two lake samples (GKS, KAI) only a comparably slight anthropogenic influence could be detected (Table 3).

3.3. Isolation of pure cultures

In total, 264 isolates (see Supplementary 2) were successfully cultivated and grown in pure cultures with the methods described. Morphological distinction was observed within but not between sampling sites. 31.82 % of the isolates were identified as gram-positive.

180 bacterial colonies showed visible growth at 20 °C on R2A and MH agar. 84 out of the initial 264 bacterial colonies grew at 4 °C on both nutrition media.

3.4. Agar disk diffusion test

Out of 264 bacterial isolates tested for antibiotic susceptibility, 54.92 % were MDR and 17.80 % of the bacterial isolates could successfully be inhibited in their growth by all antibiotics tested (Fig. 2). The percentage of resistant isolates was the lowest against Linezolid and Gentamicin and the highest in Trimethoprim and Ampicillin (see Table 4).

Overall, AR bacteria were isolated from all habitats but one (Hafelekar) with Gentamicin being the most effective antibiotic throughout all sampling sites. On average, AR was higher in cryospheric samples (snow and ice) than in freshwater ecosystems.

The highest percentage of MDR microorganisms were isolated from

Table 2

The relative level of anthropogenic influence on glacier samples. Determination was based on colored heatmaps (Strava) highlighting human activities. This allowed the classification of the sampling site based on the intensity of direct human presence as well as additional information. Samples from Tux Glacier were mostly taken within the touristic ice cave NIP.

Site	Direct human presence			Additional Info	Relative level of anthropogenic influence
	Low	Medium	High		
Jamtal Glacier	40 %	2 %	0 %	No skiing area	Low
Stubai Glacier	21 %	21 %	28 %	Skiing area	Medium
Tux Glacier	20 %	22 %	36 %	50,000 visitors/year	High

Table 1

Overview of the antibiotics used including general information about them and concentration of the filter disks used for the agar disk diffusion test.

Antibiotic	Class	Concentration	AWaRe group	Synthesis
Ampicillin	Beta-lactam	2 µg	Access	semisynthetic
Chloramphenicol	–	30 µg	Access	semisynthetic
Gentamicin	Aminoglycoside	10 µg	Access	natural
Linezolid	Oxazolidinone	10 µg	Reserve	synthetic
Nitrofurantoin	Nitrofuran	100 µg	Access	synthetic
Novobiocin	Aminocoumarine	5 µg	Access	natural
Trimethoprim	Diaminopyrimidine	5 µg	Access	synthetic
Vancomycin	Glycopeptide	5 µg	Watch	natural

Table 3
Anthropogenic influence on snow and water samples based on CLC data and additional info available. Water quality was derived from Tiris Maps provided by the Amt der Tiroler Landesregierung (2023). Blue = lake samples, yellow = river samples, red = non-glacial snow samples.

Site	Anthropogenically used area* (%)	Additional Info	Relative level of anthropogenic influence
BAR	95.7	Public swimming lake	high
GKS	0	High altitude lake	low
KAI	0	Skiing area	low
WSL	42.3	Public swimming lake	medium
Fishpond	35.78	Pisciculture	medium
Inn	48.7	Water quality = 3	medium
Jambach	17.9	Water quality = 1.67	low
Melach	31.8	Water quality = 2	low
Sanna	36.1	Water quality = 5	medium
Trisanna	34.3	Water quality = 3	medium
Zirmbach	64.2	Water quality = 2	medium
Ellmau	100	Gas station	high
Falzthurn	6,09	Mountain area	low
Hafelekar	0 %	Mountain top	low
Hungerburg	33.42	Skiing area	medium
KAI	38.9	Skiing area	medium
Largoz	0	Mountain area	low
Seegrube	100	Skiing area	high
BGS	0	Mountain area	low

snow and ice samples at BGS (100 %), gas station Ellmau (100 %), and Tux Glacier (95.24 %) as well as from freshwater samples of two swimming lakes (BAR and WSL (71.43 %)) and two rivers (Melach and Trisanna (60 %)). These sites also showed the highest numbers of AR against all tested antibiotics (Fig. 3).

Contrary, no MDR bacteria have been isolated from snow sampled at Hafelekar and from freshwater samples taken at GKS and KAI.

We observed that the nature of antibiotics (synthetic, semisynthetic, or natural) did not have a significant effect on their overall effectiveness against environmental bacterial isolates ($p = 0.05$, Kruskal-Wallis).

3.5. Anthropogenic influence

The number of AR bacteria was significantly higher in areas with more anthropogenic influence ($p < 0.001$). Further analysis using a post hoc test indicated a significant difference in the presence of AR bacteria between low, medium, and high levels of human impact. AR in highly-impacted sampling sites was on average 18.5 % higher than in medium-impacted areas and 29.8 % higher than in regions with a comparably low anthropogenic influence.

The comparison of similar habitats confirmed the overall trend except for the following differences. The presence of AR in bacterial isolates from glacier habitats was significantly affected by the level of anthropogenic influence ($p < 0.01$). However, this effect was not evident when comparing sampling sites from medium to high levels of human influence ($p = 0.61$).

The anthropogenic impact also had a significant effect on AR in snow samples ($p < 0.01$), though a Tukey post hoc test indicated that there was no significant difference between areas with low and medium influence.
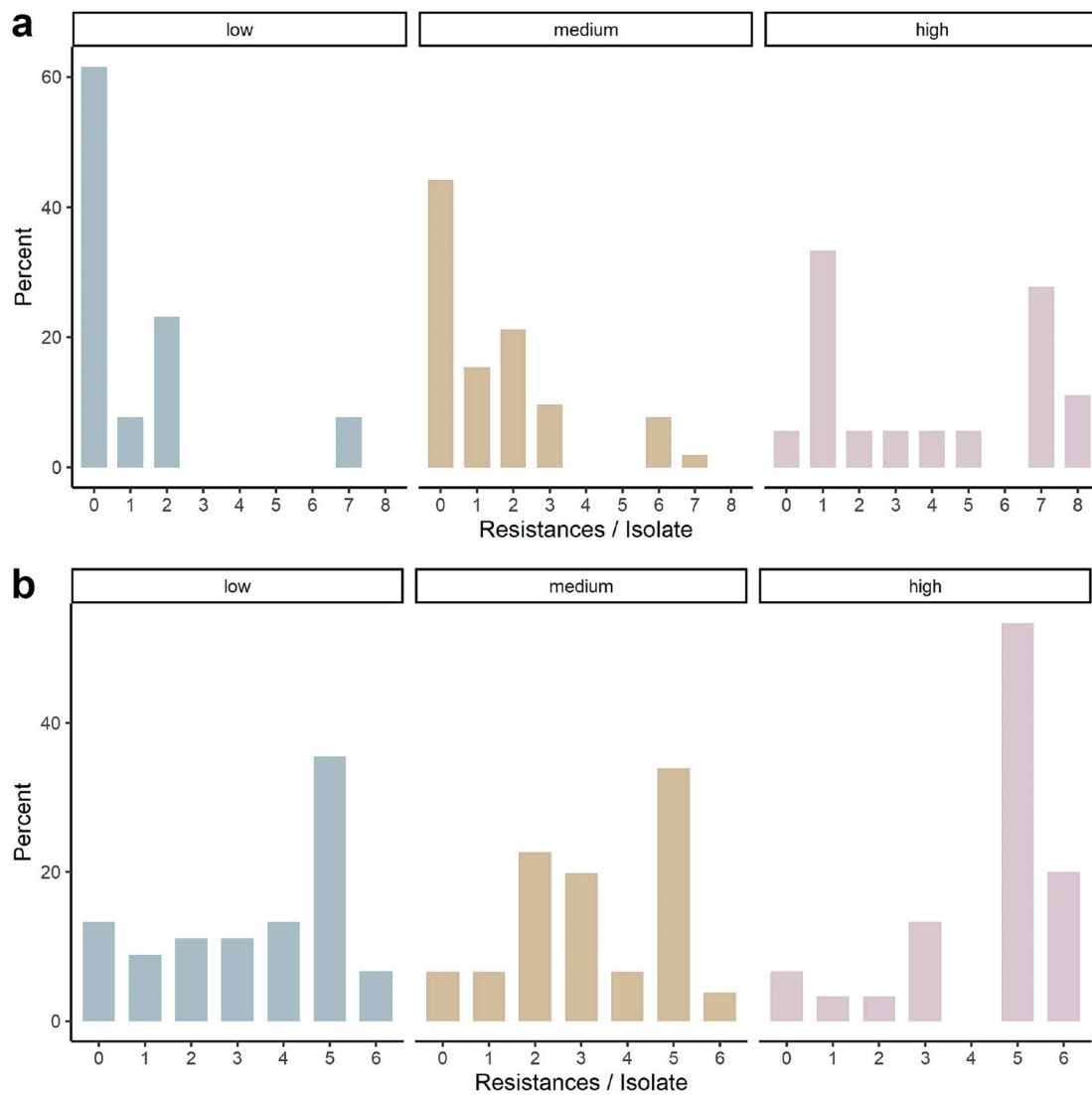
Fig. 2. Distribution of the number of AR within a single bacterial isolate in low, medium, and highly influenced sampling sites. Gram-positive bacteria (a) were tested against 8 different antibiotics including linezolid and vancomycin whereas gram-negative isolates (b) were tested for antibiotic resistance against 6 antimicrobial substances.

Table 4
Overview of resistant isolates (gram-positive and gram-negative) found against each of the 8 antibiotics. AMP = Ampicillin, C = Chloramphenicol, CN = Gentamicin, LZD = Linezolid, F = Nitrofurantoin, W = Trimethoprim, VA = Vancomycin, * = not effective against gram-negative bacteria.

	Antibiotic	AMP	C	CN	LZD*	F	NV	W	VA*
Gram-positive	Resistant Isolates	29	18	10	13	23	19	33	18
	Resistant Isolates [%]	34,52	21,43	11,90	15,48	27,38	22,62	39,29	21,43
Gram-negative	Resistant Isolates	130	77	33	–	121	137	132	–
	Resistant Isolates [%]	77,22	42,78	18,33	–	67,22	76,11	73,33	–

Lake samples significantly (p < 0.001; Kruskal-Wallis) differed regarding AR across varying levels of anthropogenic influence. Conversely, AR did not vary significantly between river samples from areas with low and medium levels of anthropogenic influence (*p* = 0.051; with Kruskal-Wallis). No river samples with high human impact were present (Fig. 4).

3.6. Sequencing

The average length of the trimmed sequences was 590 bp. A successful BLAST search could be performed in 214 sequences. *Pseudomonadaceae* was identified as the most abundant bacterial family throughout all samples corresponding to approximately 29 % of all sequenced isolates. Other abundant families include *Bacillaceae* (~16 %), *Oxalobacteraceae* (~11 %), *Flavobacteriaceae* (10 %), and *Micrococcaceae* (10 %). At species level, we observed a relatively high diversity within *Pseudomonadaceae* with 21 different species while species diversity was lower within other highly abundant bacterial families.

The two opportunistic pathogens *Escherichia coli* (BAR), and *Enterobacter cloacae* (WSL) have been isolated from swimming lake samples. Both species were MDR and showed enhanced resistance against Trimethoprim, Novobiocin, and Ampicillin (*E. coli*) as well as Nitrofurantoin (*E. cloacae*). On the other hand, the four different *Psychrobacter* species were only found in snow and ice samples and were resistant to up
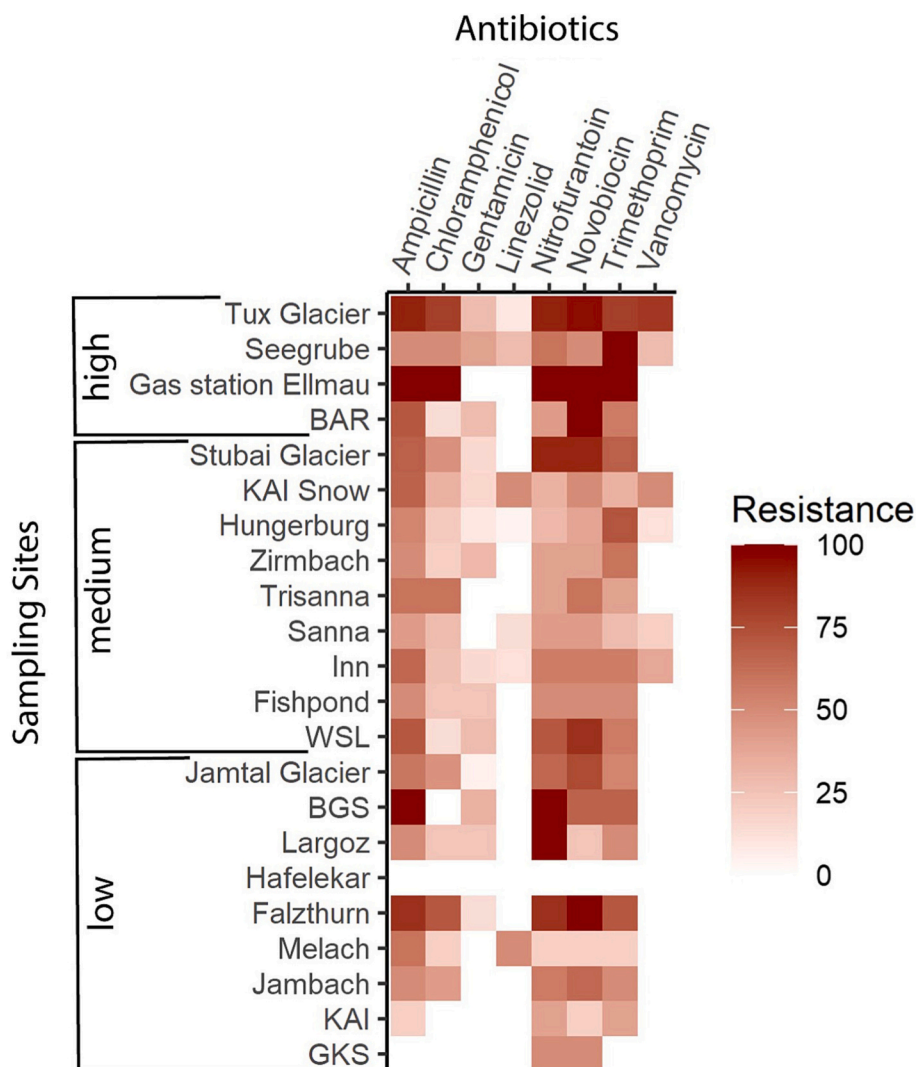
Fig. 3. Heatmap illustrating the distribution of AR throughout the different sampling sites while considering the anthropogenic influence in the respective environments.

to four antibiotics.

Janthinobacterium, a psychrophilic genus, was isolated from a variety of sampling sites including glacier ice, seasonal snow, high-altitude lakes, and flowing waters, and showed different susceptibility to the antibiotics depending on the sampling site.

4. Discussion

Antimicrobial resistance is a major global threat to human health (WHO Report, 2023), however, we still lack knowledge when it comes to AR in environmental samples.

In this study, we investigated the antibiotic susceptibility of culturable bacterial strains obtained from cryospheric and freshwater environments under different levels of anthropogenic influence. The aim was to explore the correlation between resistance and direct human presence to help closing this existing knowledge gap. Here, the Kirby-Bauer test allows for a comprehensive analysis of existing phenotypic resistance of culturable microorganisms. Although metagenomic or high-throughput approaches could further enhance the understanding of AR in environmental samples, cultivation-based approaches offer manifold advantages such as being well-proven, identifying resistance mechanisms with unknown ARGs, showing the actual viability of bacterial strains in the presence of antibiotics, and being fast and reliable.

4.1. Sequencing

Pseudomonadaceae emerged as the predominant bacterial family across all samples, constituting approximately 29 % of all sequenced isolates. This prevalence underscores the significance of Pseudomonas species in these environments, which are renowned for their intrinsic resistance mechanisms such as 12- to 100-fold reduced outer membrane permeability (Hancock and Brinkman, 2002), efflux pumps, and antibiotic-inactivating enzymes (Pang et al., 2019).

Overall, the culturable microbial community throughout all samples was dominated by gram-negative bacteria. A unique cell wall structure and ARGs like AmpC make many of these bacteria inherently resistant to some antibiotics like beta-lactam (Poole, 2011; Impey et al., 2020). This likely also contributes to the high numbers of AR found within the group of gram-negative bacteria compared to the gram-positive isolates (Table 4).

Bacterial species that are closely associated with humans, such as *Escherichia coli* and *Enterobacter cloacae* have been found in samples collected from swimming lakes, and therefore indicate the possible contamination of freshwater resources by direct anthropogenic influence. Both bacteria, isolated from medium and high anthropogenically influenced sampling sites, were found to be MDR. Antibiotic resistance in *E. coli* has become a challenge in clinical environments recently
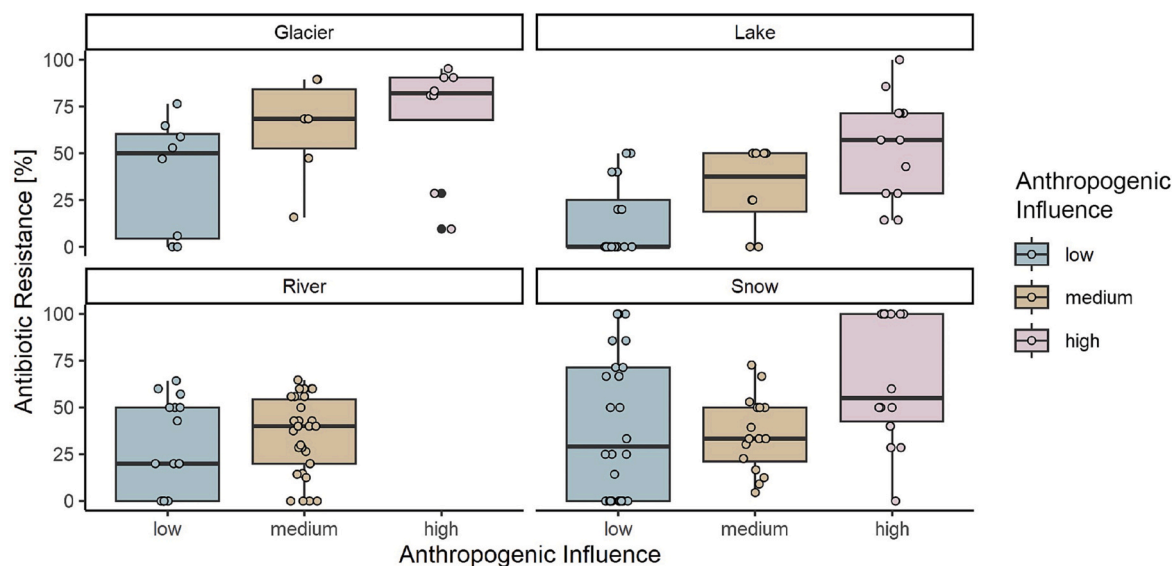
Fig. 4. Boxplot showing the difference in the percentage of AR bacteria in dependence of the anthropogenic influence. The samples were split up based on their origin (glacier, lake, river, snow). This increases comparability since human impact was determined on a relative basis with different methods for different sample types.

despite it being intrinsically susceptible to most relevant antibiotics (Poirel et al., 2018).

On the other hand, the identification of Psychrobacter species exclusively in snow and ice samples underscores their psychrophilic character, thriving in the extreme cold conditions characteristic of cryospheric environments.

However, since this sequencing was performed by culturable isolates from a variety of sampling sites, this does not reflect an accurate overview of the microbial composition of these habitats.

4.2. Agar disk diffusion test

Out of all successfully grown isolates (264), only 17.80 % were susceptible to all antibiotics tested. In contrast, 26.14 % of the bacterial isolates showed increased tolerance to five different antibiotics and over 50 % were MDR. This indicates the limited effectiveness of these eight antibiotics against the majority of the environmental bacterial strains analyzed here. Studies focusing on AR in soil have also shown that organisms can exhibit intrinsic MDR phenotypes to a variety of antibiotics independent of the antimicrobial nature (Dantas et al., 2008; D'Costa et al., 2006).

In general, AR is a highly complex topic especially when it comes to natural environments (Wright, 2007) because environmental bacteria harbor a significant prevalence of intrinsic resistance mechanisms like reduced outer membrane permeability or efflux pumps (Cox and Wright, 2013). This intrinsic resistance is likely contributing to a portion of the resistance found in this study. Additionally, AR organisms and antibiotics from anthropogenic sources that enter natural habitats through various sources can increase the selective pressure on indigenous bacteria (Baquero et al., 2008) which is a phenomenon not limited to habitats with proximity to human settlements (e.g. McCann et al., 2019; Depta and Niedźwiedzka-Rystwej, 2023). Previous studies revealed that specific AR mechanisms (e.g. vancomycin resistance) occurred long before the discovery of the first antimicrobial (D'Costa et al., 2011), emphasizing the complexity of AR in natural environments.

Although the cultivation-based approach used in this study does not allow to identify the original source for AR, a combination of intrinsic and acquired resistance seems most likely.

Resistance rates varied among the tested antibiotics. For example, Linezolid, a synthetic antibiotic classified as "Reserve" in the WHO AWaRe list, was the most active with only low levels of AR (15.66 %). It

is considered an effective last-resort antibiotic to treat a variety of gram-positive infections (Theil et al., 2020). However, concerns about the rise of resistance against oxazolidinones are supported by recent studies in clinical (Bender et al., 2018), and even environmental isolates (Fioriti et al., 2021). Our results support these findings, as they reveal the presence of a certain level of linezolid resistance in environmental cryospheric samples.

Gentamicin and vancomycin also showed relatively high effectiveness in inhibiting the growth of isolated bacteria. Nevertheless, resistance to either of these antibiotics was prevalent in some bacterial strains in most of the sampling sites. ARGs that are widespread in environmental microbial communities, could be responsible for the observed gentamicin (Heuer et al., 2002) and vancomycin resistance (Guardabassi and Agersø, 2006).

Trimethoprim, ampicillin, and novobiocin demonstrated lower effectiveness, indicating a higher prevalence of resistance mechanisms against these drugs within the tested bacterial strains. The number of resistant isolates against broad-spectrum antibiotics like chloramphenicol was similar to other natural habitats like the Arctic (Mogrovejo et al., 2020) and Antarctic (Tam et al., 2015).

Resistances against all antibiotics were found independent of their nature (natural, semisynthetic, synthetic). Particularly, reduced susceptibility to synthetic antibiotics supports the assumption that AR in the environment is, to some extent, acquired and influenced by humans.

4.3. Antibiotic resistance and the anthropogenic influence

The results of our study offer compelling evidence of the substantial impact of the anthropogenic influence on AR in the examined environments. We observed a significant increase in AR and MDR bacteria when human impact was high. Similar results have been reported by other studies looking into the effect of human impact on sediment (Bhattacharyya et al., 2019), rivers (Guan et al., 2022), tropical (Pontes et al., 2009), and polar regions (Tan et al., 2018).

The long-established role of anthropogenic (mis-)use of antibacterial medication as a major driver for the development of AR in clinical settings (Austin et al., 1999; Kolár et al., 2001) is complemented by the drastic increase of the selective pressure on bacteria in natural environments due to mass production of antibiotics (Larsson, 2014; Tello et al., 2012; Xiong et al., 2015). Moreover, the enrichment and persistence of antimicrobial resistance can occur even at low antibiotic

concentrations (Gullberg et al., 2011) frequently encountered in natural environments.

A variety of mechanisms (e.g. horizontal gene transfer) can lead to the spread of ARGs and subsequent AR in the environment (Skandalis et al., 2021) potentially even leading to the transfer of resistances back to previously susceptible pathogenic bacteria in clinical settings (Maeusli et al., 2020; Wright, 2010).

The comparisons of similar environmental samples with varying levels of anthropogenic influence showed a substantial difference in the prevalence of AR bacteria.

Here, human impact can be exemplified by sampling sites like the NIP, where individuals often come into direct skin contact with snow and ice surfaces, and therefore potentially introducing and spreading (AR) microorganisms.

Similarly, the high number of frequent visitors of ski resorts at Stubai Glacier or Seegrube may also contribute to the dispersion of AR bacteria.

Furthermore, the swimming lakes WSL and BAR are both examples of water sampling sites with extensive direct human contact and therefore an increased likelihood of the spread of potential AR bacteria into freshwater ecosystems.

Highly-impacted cryospheric sampling sites like 'Gas Station Ellmau', had a significantly higher proportion of AR bacteria when compared to regions with low anthropogenic influence ($p < 0.001$).

This trend also remained consistent when focusing on glacier habitats. The uniqueness of Tux Glacier, being an ice cave open to the public with limited ventilation, led to a notably higher number of AR isolates compared to other glacier samples. Among the antibiotics tested, only Linezolid (<10 % resistant bacteria) and Gentamicin (<30 % resistant bacteria) proved to be effective antibiotics at this sampling site. Additionally, antibiotic resistance rates were among the highest across all sampling sites with 95 % of the isolates tested being MDR and > 80 % of the isolated bacteria being resistant to at least one of the other six antibiotics. Surprisingly, vancomycin was ineffective in 83.33 % of the tests which is comparably higher than reports from other glaciers (Segawa et al., 2013) and even hospital effluents (Nuñez et al., 2016).

In comparison, to the medium (Stubai) and highly influenced (Tux) glaciers, Jamtal Glacier lacks skiing infrastructure and is consequently less exposed to human activities. As a result, a significantly lower number of antibiotic-resistant microbes and MDR strains have been observed, suggesting that direct human presence is an important factor in the dissemination of AR in cryospheric habitats. This assumption is also supported by the susceptibility of respective bacterial isolates to the clinically relevant last-line-of-defense antibiotics linezolid and vancomycin.

Results of bacterial isolates sampled at glaciers in the Tyrolean Alpine space emphasize the significance of the anthropogenic influence on cold habitats which aligns with results from human-inhabited areas in Antarctica (Miller et al., 2009).

However, direct human impact represents just one facet among various factors influencing AR in cold habitats. The transport of ARGs through biological and physical mechanisms (Allen et al., 2010) presents a plausible explanation for the presence of AR bacteria in relatively remote regions, as observed in this study. Additionally, intrinsic resistance, arising from non-specific cellular processes not originally designed to protect bacteria from antibacterial substances (Tamae et al., 2008), possibly explains some of the identified resistances.

Freshwater samples showed a similar overall trend as snow and ice samples. Higher levels of anthropogenic influence corresponded to more MDR isolates and significantly higher numbers of overall AR. BAR and WSL, two popular swimming lakes in Tyrol largely surrounded by agricultural and urbanized areas and with substantial direct human contact, serve as examples of highly influenced sampling sites. Compared to regions with lower anthropogenic influence, such as the high alpine lakes GKS and KAI, the swimming lakes, BAR, and WSL, hosted a significantly higher number of culturable MDR bacteria. Notably, the two MDR opportunistic pathogens, *Escherichia coli* and

Enterobacter cloacae, were isolated from these swimming lakes that have medium and high direct human impact. This underscores the intricate relationship between anthropogenic activities and the emergence of antibiotic resistance in environmental settings. The observed resistance of the *E. coli* strain against three different antibiotics highlights its capacity to accumulate ARGs despite being intrinsically susceptible to most antibiotics (Poirel et al., 2018). *E. cloacae* on the other hand is intrinsically resistant to Ampicillin (Davin-Regli and Pagès, 2015). Thus, our results support the concerns that it could potentially become an emerging global threat due to the rise in MDR strains (Annavajhala et al., 2019).

While the trend of increased numbers of AR and MDR at sampling sites with higher human influence was evident for lake samples, no significant difference could be observed in river ecosystems. However, it is essential to note that none of the studied rivers was categorized as strongly influenced by humans (based on urbanized and agricultural areas in their disposal areas).

5. Conclusion

In this study, we investigated AR in environmental cryospheric and freshwater samples and explored the correlation between resistance and direct human presence. Our findings shed new light on the significant impact of anthropogenic influence on AR in these habitats.

The Kirby-Bauer test revealed a concerning prevalence of culturable resistant bacterial isolates from various cryospheric environments. >50 % of MDR microorganisms indicate limited effectiveness of the tested antibiotics against the majority of environmental bacterial strains. Even so-called reserve antibiotics like linezolid were ineffective against some isolates (15.66 %) which supports the concerns about the decreased susceptibility against oxazolidinones.

Antibiotics, as key players, are essential for modern medicine, and their efficacy is threatened by the increasing prevalence of AR not only in clinical settings but also in environmental samples.

Furthermore, the results from our study emphasize the significance of the anthropogenic influence on AR. A trend towards reduced antibiotic susceptibility with increased human activity was observed even in cold habitats. This correlation was evident in glacier samples as well as seasonal snow and lake habitats.

Climate change-induced glacier melting possibly creates a pathway for AR bacterial strains to come in contact with urbanized areas and human activities. Transferring associated ARGs to pathogenic phenotypes through known mechanisms like horizontal gene transfer (Maeusli et al., 2020; Rhodes et al., 2000; Wright, 2010), could further intensify the rising problem of AR.

Therefore, our study highlights the urgent need to comprehensively monitor the spread of AR in natural and extreme environments like the cryosphere. Future research could further investigate non-culturable bacteria with a metagenomic approach to gain an even better understanding of AR in cryospheric habitats and the impact of anthropogenic influence on it.

Overall, our study contributes valuable insights into the complex dynamics of AR in environmental samples in the Tyrolean Alpine space and underlines the critical role of the human impact in shaping resistance patterns. Gaining insight into these factors and comprehending how resistant microorganisms spread in these habitats is crucial for understanding the extent of this global phenomenon.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171532.

Autonomous Province of Trento (Accordo di Programma P2211003I to TW).

References

Alcock, Brian P., Huynh, William, Chalil, Romeo, Smith, Keaton W., Raphenya, Amogelang R., Wlodarski, Mateusz A., et al., 2023. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the comprehensive antibiotic resistance database. Nucleic Acids Res. 51 (D1), D690–D699. https://doi.org/10.1093/nar/gkac920.

Allen, Heather K., Donato, Justin, Wang, Helena Huimi, Cloud-Hansen, Karen A., Davies, Julian, Handelsman, Jo, 2010. Call of the wild: antibiotic resistance genes in natural environments. Nat. Rev. Microbiol. 8 (4), S. 251–259. https://doi.org/10.1038/nrmicro2312.

Amt der Tiroler Landesregierung, 2023. Tiris Maps. Retrieved from: https://maps.tirol.gv.at/.

Annavajhala, Medini K., Gomez-Simmonds, Angela, Uhlemann, Anne-Catrin, 2019. Multidrug-resistant enterobacter cloacae complex emerging as a global, diversifying threat. Front. Microbiol. 10, S. 44. https://doi.org/10.3389/fmicb.2019.00044.

Austin, D.J., Kristinsson, K.G., Anderson, R.M., 1999. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proc. Natl. Acad. Sci. U. S. A. 96 (3), S. 1152–1156. https://doi.org/10.1073/pnas.96.3.1152.

Baquero, Fernando, Martínez, José-Luis, Cantón, Rafael, 2008. Antibiotics and antibiotic resistance in water environments. Curr. Opin. Biotechnol. 19 (3), S. 260–265. https://doi.org/10.1016/j.copbio.2008.05.006.

Bender, Jennifer K., Cattoir, Vincent, Hegstad, Kristin, Sadowy, Ewa, Coque, Teresa M., Westh, Henrik, et al., 2018. Update on prevalence and mechanisms of resistance to linezolid, tigecycline and daptomycin in enterococci in Europe: towards a common nomenclature. Drug resistance updates: reviews and commentaries in antimicrobial and anticancer chemotherapy 40, S. 25–39. https://doi.org/10.1016/j.drup.2018.10.002.

Bhattacharyya, Roby P., Bandyopadhyay, Nirmalya, Ma, Peijun, Son, Sophie S., Liu, Jamin, He, Lorrie L., et al., 2019. Simultaneous detection of genotype and phenotype enables rapid and accurate antibiotic susceptibility determination. Nat. Med. 25 (12), S. 1858–1864. https://doi.org/10.1038/s41591-019-0650-9.

Chao, Kuan-Hao, Barton, Kirston, Palmer, Sarah, Lanfear, Robert, 2021. sangeranalyseR: simple and interactive processing of sanger sequencing data in R. Genome Biol. Evol. 13, 3. https://doi.org/10.1093/gbe/evab028.

Cox, Georgina, Wright, Gerard D., 2013. Intrinsic antibiotic resistance: mechanisms, origins, challenges and solutions. International journal of medical microbiology: IJMM 303 (6–7), S. 287–292. https://doi.org/10.1016/j.ijmm.2013.02.009.

Dantas, Gautam, Sommer, Morten O.A., Oluwasegun, Rantimi D., Church, George M., 2008. Bacteria Subsisting on Antibiotics. Science (New York, N.Y.), vol. 320 (5872), pp. S. 100–103. https://doi.org/10.1126/science.1155157.

Davin-Regli, Anne, Pagès, Jean-Marie, 2015. Enterobacter aerogenes and Enterobacter cloacae; versatile bacterial pathogens confronting antibiotic treatment. Front. Microbiol. 6, S. 392. https://doi.org/10.3389/fmicb.2015.00392.

D'Costa, Vanessa M., McGrann, Katherine M., Hughes, Donald W., Wright, Gerard D., 2006. Sampling the Antibiotic Resistome. Science (New York, N.Y.), vol. 311 (5759), pp. S. 374–377. https://doi.org/10.1126/science.1120800.

D'Costa, Vanessa M., King, Christine E., Kalan, Lindsay, Morar, Mariya, Sung, Wilson W. L., Schwarz, Carsten, et al., 2011. Antibiotic resistance is ancient. Nature 477 (7365), S. 457–461. https://doi.org/10.1038/nature10388.

Depta, Julia, Niedźwiedzka-Rystwej, Paulina, 2023. The phenomenon of antibiotic resistance in the polar regions: an overview of the global problem. Infection and drug resistance 16, S. 1979–1995. https://doi.org/10.2147/IDR.S369023.

European Union, 2018. Copernicus land monitoring service 2018, European Environment Agency (EEA). Corine Land Cover (CLC) 2018, Version 2020_20u1. Available at: https://land.copernicus.eu/pan-european/corine-land-cover/clc2018.

Fioriti, Simona, Coccitto, Sonia Nina, Cedraro, Nicholas, Simoni, Serena, Morroni, Gianluca, Brenciani, Andrea, et al., 2021. Linezolid resistance genes in enterococci isolated from sediment and zooplankton in two Italian coastal areas. Appl. Environ. Microbiol. 87 (9) https://doi.org/10.1128/AEM.02958-20.

Gattinger, Daniel, Pichler, Katrin, Weil, Tobias, Sattler, Birgit, 2023. A comparative approach to confirm antibiotic-resistant microbes in the cryosphere. Front. Microbiol. 14, S. 1212378. https://doi.org/10.3389/fmicb.2023.1212378.

Guan, Yongjing, Jia, Jia, Fan, Xiaoteng, Li, Kaiqi, Wang, Zaizhao, 2022. Anthropogenic impacts on antibiotic resistance genes and their hosts from pristine to urban river using metagenomic and binning approaches. Aquatic toxicology (Amsterdam, Netherlands) 249, S. 106221. https://doi.org/10.1016/j.aquatox.2022.106221.

Guardabassi, Luca, Agersø, Yvonne, 2006. Genes homologous to glycopeptide resistance vanA are widespread in soil microbial communities. FEMS Microbiol. Lett. 259 (2), S. 221–225. https://doi.org/10.1111/j.1574-6968.2006.00270.x.

Gullberg, Erik, Cao, Sha, Berg, Otto G., Ilbäck, Carolina, Sandegren, Linus, Hughes, Diarmaid, Andersson, Dan I., 2011. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog. 7 (7), e1002158 https://doi.org/10.1371/journal.ppat.1002158.

Hancock, Robert E.W., Brinkman, Fiona S.L., 2002. Function of pseudomonas porins in uptake and efflux. Annu. Rev. Microbiol. 56, S. 17–38. https://doi.org/10.1146/annurev.micro.56.012302.160310.

Harrington, B.J., Gaydos, J.M., 1984. Five-hour novobiocin test for differentiation of coagulase-negative staphylococci. J. Clin. Microbiol. 19 (2), S. 279–280. https://doi.org/10.1128/jcm.19.2.279-280.1984.

Hernández, Jorge, González-Acuña, Daniel, 2016. Anthropogenic antibiotic resistance genes mobilization to the polar regions. Infection Ecology & Epidemiology 6, S. 32112. https://doi.org/10.3402/iee.v6.32112.

Heuer, H., Krögerrecklenfort, E., Wellington, E.M.H., Egan, S., van Elsas, J.D., van Overbeek, L., et al., 2002. Gentamicin resistance genes in environmental bacteria: prevalence and transfer. FEMS Microbiol. Ecol. 42 (2), S. 289–302. https://doi.org/10.1111/j.1574-6941.2002.tb01019.x.

Hooper, Sarah E., Weller, Hannah, Amelon, Sybill K., 2020. Countcolors, an R package for quantification of the fluorescence emitted by Pseudogymnoascus destructans lesions on the wing membranes of hibernating bats. J. Wildl. Dis. 56 (4), S. 759–767. https://doi.org/10.7589/2019-09-231.

Impey, Rachael E., Hawkins, Daniel A., Sutton, J. Mark, Costa, Da Soares, Tatiana, P., 2020. Overcoming intrinsic and acquired resistance mechanisms associated with the Cell Wall of gram-negative Bacteria. Antibiotics 9 (9). https://doi.org/10.3390/antibiotics9090623.

Jungblut, Anne D., Velazquez, David, Cirés, Samuel, Kleinteich, Julia, Padinchati, Kottekkatu, Krishnan, Sattler, Birgit, Comte, Jérôme, 2022. Editorial: digitizing frozen earth-revealing microbial diversity and physiology in the cryobiosphere through "omics" tools, volume II. Front. Microbiol. 13, S. 1013398. https://doi.org/10.3389/fmicb.2022.1013398.

Kolár, M., Urbánek, K., Látal, T., 2001. Antibiotic selective pressure and development of bacterial resistance. Int. J. Antimicrob. Agents 17 (5), S. 357–363. https://doi.org/10.1016/s0924-8579(01)00317-x.

Laborda, Pablo, Sanz-García, Fernando, Ochoa-Sánchez, Luz Edith, Gil-Gil, Teresa, Hernando-Amado, Sara, Martínez, José Luis, 2022. Wildlife and antibiotic resistance. Front. Cell. Infect. Microbiol. 12, S. 873989. https://doi.org/10.3389/fcimb.2022.873989.

Laganà, Pasqualina, Caruso, Gabriella, Corsi, Ilaria, Bergami, Elisa, Venuti, Valentina, Majolino, Domenico, et al., 2019. Do plastics serve as a possible vector for the spread of antibiotic resistance? First insights from bacteria associated to a polystyrene piece from King George Island (Antarctica). Int. J. Hyg. Environ. Health 222 (1), S. 89–100. https://doi.org/10.1016/j.ijheh.2018.08.009.

Land Tirol, 2023. Digital Elevation Model (DGM) of Tyrol, 5m resolution. Retrieved from: https://www.data.gv.at/katalog/dataset/land-tirol_tirolgelnde#resources.

Larsson, D.G. Joakim, 2014. Antibiotics in the environment. Ups. J. Med. Sci. 119 (2), S. 108–112. https://doi.org/10.3109/03009734.2014.896438.

Maeusli, Marlène, Lee, Bosul, Miller, Sarah, Reyna, Zeferino, Lu, Peggy, Yan, Jun, et al., 2020. Horizontal Gene Transfer of Antibiotic Resistance from *Acinetobacter baylyi* to

Escherichia coli on Lettuce and Subsequent Antibiotic Resistance Transmission to the Gut Microbiome. mSphere 5 (3). https://doi.org/10.1128/mSphere.00329-20.

McCann, Clare M., Christgen, Beate, Roberts, Jennifer A., Su, Jian-Qiang, Arnold, Kathryn E., Gray, Neil D., et al., 2019. Understanding drivers of antibiotic resistance genes in high Arctic soil ecosystems. Environ. Int. 125, 497–504. https://doi.org/10.1016/j.envint.2019.01.034.

Miller, Robert V., Gammon, Katharine, Day, Martin J., 2009. Antibiotic resistance among bacteria isolated from seawater and penguin fecal samples collected near Palmer Station. Antarctica. Canadian journal of microbiology 55 (1), S. 37–45. https://doi.org/10.1139/W08-119.

Mogrovejo, Diana C., Perini, Laura, Gostinčar, Cene, Sepčić, Kristina, Turk, Martina, Ambrožič-Avguštin, Jerneja, et al., 2020. Prevalence of antimicrobial resistance and hemolytic phenotypes in Culturable Arctic Bacteria. Front. Microbiol. 11, S. 570. https://doi.org/10.3389/fmicb.2020.00570.

Nuñez, Lidia, Tornello, Carina, Puentes, Noel, Espigares Rodríguez, Elena, Moreno Roldán, Elena, Espigares García, Miguel, Moretton, Juan, 2016. Hospital effluent constitutes a source of vancomycin-resistant enterococci, pp. 2340–9894. https://doi.org/10.4321/S2340-98942016000300003.

Pang, Zheng, Raudonis, Renee, Glick, Bernard R., Lin, Tong-Jun, Cheng, Zhenyu, 2019. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnol. Adv. 37 (1), 177–192. https://doi.org/10.1016/j.biotechadv.2018.11.013.

Pawlowski, Andrew C., Wang, Wenliang, Koteva, Kalinka, Barton, Hazel A., McArthur, Andrew G., Wright, Gerard D., 2016. A diverse intrinsic antibiotic resistome from a cave bacterium. Nat. Commun. 7, S. 13803. https://doi.org/10.1038/ncomms13803.

Poirel, Laurent, Madec, Jean-Yves, Lupo, Agnese, Schink, Anne-Kathrin, Kieffer, Nicolas, Nordmann, Patrice, Schwarz, Stefan, 2018. Antimicrobial Resistance in Escherichia coli. Microbiology spectrum 6 (4). https://doi.org/10.1128/microbiolspec.ARBA-0026-2017.

Pontes, D.S., Pinheiro, F.A., Lima-Bittencourt, C.I., Guedes, R.L.M., Cursino, L., Barbosa, F., et al., 2009. Multiple antimicrobial resistance of gram-negative bacteria from natural oligotrophic lakes under distinct anthropogenic influence in a tropical region. Microb. Ecol. 58 (4), 762–772. https://doi.org/10.1007/s00248-009-9539-3.

Poole, Keith, 2011. Pseudomonas aeruginosa: resistance to the max. Front. Microbiol. 2, S. 65. https://doi.org/10.3389/fmicb.2011.00065.

Review on Antimicrobial Resistance. (2016). Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. AMR Review. Available at: https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf.

Rhodes, G., Huys, G., Swings, J., McGann, P., Hiney, M., Smith, P., Pickup, R.W., 2000. Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: implication of Tn1721 in dissemination of the tetracycline resistance determinant tet A. Appl. Environ. Microbiol. 66 (9), 3883–3890. https://doi.org/10.1128/AEM.66.9.3883-3890.2000.

Rossi, Florent, Péguilhan, Raphaëlle, Turgeon, Nathalie, Veillette, Marc, Baray, Jean-Luc, Deguillaume, Laurent, et al., 2023. Quantification of antibiotic resistance genes (ARGs) in clouds at a mountain site (puy de Dôme, Central France). Sci. Total Environ. 865, S. 161264. https://doi.org/10.1016/j.scitotenv.2022.161264.

Segawa, Takahiro, Takeuchi, Nozomu, Rivera, Andres, Yamada, Akinori, Yoshimura, Yoshitaka, Barcaza, Gonzalo, et al., 2013. Distribution of antibiotic resistance genes in glacier environments. Environ. Microbiol. Rep. 5 (1), 127–134. https://doi.org/10.1111/1758-2229.12011.

Skandalis, Nicholas, Maeusli, Marlène, Papafotis, Dimitris, Miller, Sarah, Lee, Bosul, Theologidis, Ioannis, Luna, Brian, 2021. Environmental Spread of Antibiotic Resistance. Antibiotics (Basel, Switzerland) 10 (6). https://doi.org/10.3390/antibiotics10060640.

Strava, 2023. https://www.strava.com/heatmap#7.30/10.83618/47.23078/hot/all.

Tam, Heng Keat, Wong, Clemente Michael Vui Ling, Yong, Sheau Ting, Blamey, Jenny, González, Marcelo, 2015. Multiple-antibiotic-resistant bacteria from the maritime Antarctic. Polar Biol. 38 (8), S. 1129–1141. https://doi.org/10.1007/s00300-015-1671-6.

Tamae, Cindy, Liu, Anne, Kim, Katherine, Sitz, Daniel, Hong, Jeeyoon, Becket, Elinne, et al., 2008. Determination of antibiotic hypersensitivity among 4,000 single-gene-knockout mutants of Escherichia coli. J. Bacteriol. 190 (17), S. 5981–5988. https://doi.org/10.1128/JB.01982-07.

Tan, Lu, Li, Linyun, Ashbolt, Nicholas, Wang, Xiaolong, Cui, Yuxiao, Zhu, Xiao, et al., 2018. Arctic antibiotic resistance gene contamination, a result of anthropogenic activities and natural origin. Sci. Total Environ. 621, S. 1176–1184. https://doi.org/10.1016/j.scitotenv.2017.10.110.

Tello, Alfredo, Austin, Brian, Telfer, Trevor C., 2012. Selective pressure of antibiotic pollution on bacteria of importance to public health. Environ. Health Perspect. 120 (8), S. 1100–1106. https://doi.org/10.1289/ehp.1104650.

The European Committee on Antimicrobial Susceptibility Testing, 2021. EUCAST disk diffusion test methodology, version 9.0. Available at: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/2021_manuals/Manual_v_9.0_EUCAST_Disk_Test_2021.pdf.

The European Committee on Antimicrobial Susceptibility Testing, 2023. Breakpoint tables for interpretation of MICs and zone diameters, version 13.0. Available at: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_13.0_Breakpoint_Tables.pdf.

Theil, Christoph, Schmidt-Braekling, Tom, Gosheger, Georg, Schwarze, Jan, Dieckmann, Ralf, Schneider, Kristian Nikolaus, Möllenbeck, Burkhard, 2020. Clinical use of linezolid in periprosthetic joint infections - a systematic review. Journal of bone and joint infection 6 (1), S. 7–16. https://doi.org/10.5194/jbji-6-7-2020.

Ushida, Kazunari, Segawa, Takahiro, Kohshima, Shiro, Takeuchi, Nozomu, Fukui, Kotaro, Li, Zhongqin, Kanda, Hiroshi, 2010. Application of real-time PCR array to the multiple detection of antibiotic resistant genes in glacier ice samples. J. Gen. Appl. Microbiol. 56 (1), 43–52. https://doi.org/10.2323/jgam.56.43.

Ventola, C. Lee, 2015. The antibiotic resistance crisis: part 1: causes and threats. P & T: a peer-reviewed journal for formulary management 40 (4), S. 277–283. PMID: 25859123; PMCID: PMC4378521.

World Health Organization, 2019. No Time to Wait: Securing the Future from Drug-Resistant Infections. Available at: https://cdn.who.int/media/docs/default-source/documents/no-time-to-wait-securing-the-future-from-drug-resistant-infections-en.pdf?sfvrsn=5b424d7_6&download=true.

World Health Organization, 2023. Global Research Agenda for Antimicrobial Resistance in Human Health. Available at: https://www.who.int/publications/m/item/global-research-agenda-for-antimicrobial-resistance-in-human-health.

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

Wright, Gerard D., 2010. Antibiotic resistance in the environment: a link to the clinic? Curr. Opin. Microbiol. 13 (5), S. 589–594. https://doi.org/10.1016/j.mib.2010.08.005.

Xiong, Wenguang, Sun, Yongxue, Zhang, Tong, Ding, Xueyao, Li, Yafei, Wang, Mianzhi, Zeng, Zhenling, 2015. Antibiotics, antibiotic resistance genes, and bacterial community composition in fresh water aquaculture environment in China. Microb. Ecol. 70 (2), S. 425–432. https://doi.org/10.1007/s00248-015-0583-x.