

Evaluation of antimicrobial resistance in bacterial strains isolated from Antarctica

Rubiano-Labrador Carolina^{1*}, Chavarro-Mesa, Edison¹, Acevedo-Barios, Rosa¹, Pasqualino, Jorgelina², Serna, Jose², Cristina Isabella², Tabora, Natalie² and Valiente Puche, Melanie²

¹Dirección de Ciencias Básicas. Universidad Tecnológica de Bolívar. Cartagena de Indias, Colombia, drubiano@utb.co, echavarro@utb.edu.co, racevedo@utb.edu.co

²Escuela de Ingeniería, Arquitectura & Diseño. Universidad Tecnológica de Bolívar. Cartagena de Indias, Colombia, jpasqualino@utb.edu.co, mvaliente@utb.edu.co, joserna@utb.edu.co, ntabora@utb.edu.co

Abstract– *The discovery of antibiotics transformed the treatment of bacterial infections, but their excessive use has led to the accumulation of these compounds in the environment and the spread of resistant microorganisms, even in isolated regions such as Antarctica. This study evaluated the resistance of Antarctic bacteria to five groups of antibiotics (β -lactams, cephalosporins, quinolones, macrolides and sulfonamides), considering the level of human intervention at their isolation sites. Sixteen strains were analyzed by disk diffusion antibiograms, incubating the cultures at 10°C for one week. The results showed variations in resistance depending on the location. Livingston and Deception Islands presented the highest levels of resistance, with strains UTB 117 and UTB 118 (*Pseudomonas mandelii*) and UTB 145 (*Pseudomonas antarctica*) standing out, resistant to all the antibiotics evaluated. In addition, 80% of the strains showed resistance to dicloxacillin. These findings suggest that antimicrobial resistance in Antarctica is influenced by proximity to scientific bases, human activity and pollution.*

Keywords-- *Antarctica, antibiogram, antibiotic, antimicrobial resistance, bacteria.*

I. INTRODUCTION

Antibiotics are produced by fungi or bacteria that inhibit the growth of pathogenic bacteria. Antibiotics can be divided into different groups by their mechanism of action, including bactericides (which cause the death of bacteria) and bacteriostatics (which inhibit bacterial growth), or by their chemical structures, such as β -lactams, quinolones, tetracyclines, macrolides, sulfonamides, and others [1].

β -Lactam antibiotics are characterized by having a central beta-lactam ring in their molecular structure. This type of antibiotic acts by inhibiting the synthesis of the bacterial cell wall by binding to penicillin-binding proteins, which causes cell lysis [2]. Cephalosporins are antibiotics structurally similar to β -lactams but with greater resistance to β -lactamases, which gives them a broader spectrum of action against Gram-positive and Gram-negative bacteria [3]. Quinolones are synthetic antibiotics characterized by having a fluoride group in the central ring, which inhibit bacterial topoisomerases (DNA gyrase and topoisomerase IV), preventing DNA replication and transcription [4]. Macrolides are characterized by having a macrocyclic lactone ring and block bacterial protein synthesis by binding to the 50S ribosomal subunit, interfering with the elongation of the

peptide chain, which makes them useful against Gram-positive and some Gram-negative bacteria [5]. Sulfonamides, on the other hand, are synthetic antibiotics that consist of a sulfur atom, two oxygen atoms, a nitrogen atom and a carbon side group. These antibiotics inhibit the synthesis of folic acid in bacteria, which prevents the production of nucleotides essential for cell replication [6].

Antibiotics are widely used in human and veterinary medicine. However, when consumed, only a portion of them is metabolized by the body, while the remainder is excreted through urine and feces. These drugs can enter the natural ecosystem through discharges of wastewater treatment plant effluents into surface or underground water bodies, leachates from landfills, leaks in sewers, improper storage of manure in tanks or lagoons, as well as through runoff and leaching of agricultural soils fertilized with contaminated manure. In addition, improper disposal of wastewater and other discharges contributes to their accumulation in the environment, posing a risk to ecosystems and public health [7-9].

Antibiotic residues and byproducts can accelerate the emergence of resistant bacteria and the spread of resistance genes in the environment. Although antibiotic resistance is a natural phenomenon, its impact has been aggravated by overuse and inappropriate use in humans and animals, as well as by environmental release [10]. The selective pressure of these drugs has favored the development of superbacteria, some resistant to all available antibiotics, leading to untreatable infections [11]. This crisis is particularly worrying in developing countries, where factors such as high burden of infectious diseases, poor sanitation and poverty accelerate the spread of antimicrobial resistance [12]. The presence of antibiotics has been widely reported throughout the world. However, in geographically isolated regions with low anthropogenic activity such as Antarctica, information on antibiotic resistance is limited. The study of antibiotic resistance in this type of ecosystem allows us to evaluate the impact of human activity on the spread of resistance genes and antibiotic-resistant bacteria [13]. Antarctica is the coldest, driest and windiest continent, with the highest average altitude on the planet. This continent presents extreme conditions such as aridity, freeze-thaw cycles, low humidity, UV radiation and

nutrient scarcity [14]. In recent decades, anthropogenic activity in Antarctica has increased significantly due to the expansion of scientific bases and the growing presence of tourism. Currently, more than 70 research stations operate year-round or during specific seasons, which implies an increase in the transit of personnel, infrastructure and waste. At the same time, Antarctic tourism has grown exponentially, increasing the ecological footprint and the risk of alterations in fragile ecosystems [15]. These activities represent a greater risk of spreading antibiotic-resistant bacteria on the continent. Given this scenario, it is essential to assess the extent of human contamination associated with antimicrobial resistance in these ecosystems. Therefore, the objective of this study was to evaluate the resistance of bacteria isolated from Antarctica to different groups of antibiotics and analyse their relationship with the level of human intervention at the isolation sites.

II. MATERIAL AND METHODS

A. Strains

In this study, 16 halotolerant psychrophilic bacterial strains isolated from Antarctic marine sediments in previous studies were selected to evaluate their resistance to different types of antibiotics [16-17]. The taxonomic affiliation of the strains evaluated and the level of human intervention at the isolation site are presented in Table 1.

TABLE I
TAXONOMIC AFFILIATION OF THE STRAINS EVALUATED AND LEVEL OF HUMAN INTERVENTION AT THE ISOLATION SITES

Human activity	Isolation location	Taxonomic affiliation	Code
High	Deception Island	<i>Psychrobacter cryohalolentis</i>	UTB 113
		<i>Pseudomonas antarctica</i>	UTB 145
		<i>Psychrobacter glaciei</i>	UTB 167
	Livingston Island	<i>Pseudomonas mandelii</i>	UTB 115
		<i>Pseudomonas mandelii</i>	UTB 117
		<i>Pseudomonas mandelii</i>	UTB 118
Medium	Artigas Base	<i>Psychrobacter cryohalolentis</i>	UTB 170
		<i>Psychrobacter glaciei</i>	UTB 171
	Horseshoe Island	<i>Psychrobacter glaciei</i>	UTB 173
		<i>Psychrobacter glaciei</i>	UTB 174
	Half Moon Island	<i>Psychrobacter nivimaris</i>	UTB 160
	Elephant Point	<i>Oceanisphaera marina</i>	UTB 169
		<i>Oceanisphaera marina</i>	UTB 172
Low	Byers Peninsula	<i>Janthinobacterium svalbardensis</i>	UTB 106
		<i>Pseudomonas antarctica</i>	UTB 109
		<i>Exiguobacterium sp.</i>	UTB 168

B. Antibiotics

Five groups of antibiotics were evaluated in this study: β -lactams, cephalosporins, quinolones, macrolides and sulfonamides. Table 2 presents the selected antibiotics, and the concentrations evaluated.

TABLE II
SELECTED ANTIBIOTICS AND CONCENTRATION EVALUATED

Group	Antibiotic	Concentration
β -lactams	Penicillin	100 UI
	Dicloxacillin	50 mg/mL
	Amoxicillin	50 mg/mL
	Ampicillin	50 mg/mL
Cephalosporins	Cephalexin	50 mg/mL
Sulfonamides	Sulfamethazol	50g/mL

C. Antibiotic resistance evaluation

The susceptibility of the bacterial strains isolated from Antarctica to different groups of antibiotics was assessed using the disk diffusion technique on LB agar [18]. The strains were grown in LB broth and incubated at 10 °C for one week. The bacterial suspension was evenly inoculated onto LB agar plates, ensuring a homogeneous distribution of the inoculum. Antibigrams were performed using filter paper disks impregnated with 20 μ L of each antibiotic tested (Fig. 1). The media were incubated at 10 °C for 8 days. All tests were performed duplicate. After the incubation time, the diameters of the inhibition zones were measured. Growth around the filter was interpreted as resistance to the antibiotic, while the formation of an inhibition halo indicated susceptibility.

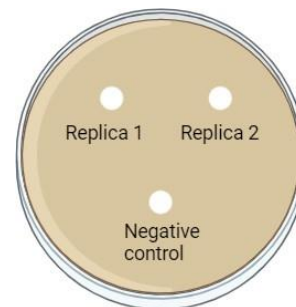


Fig.1 Scheme for the evaluation of antibiotic resistance

III. RESULTS

Bacterial strains isolated from different Antarctic locations, including Deception Island, Livingston Island, Artigas Base, Herradura Island, Media Luna Island, Elephant Point, and Byers Peninsula, were evaluated for resistance to six

antibiotics. The antibiotics evaluated were penicillin (PNC), dicloxacillin (DLX), amoxicillin (AMX), ampicillin (AMP), cephalexin (CEF), and sulfamethazole (SUF). The results show variability in resistance profiles between different strains and locations. In general, *Psychrobacter glaciei* UTB 173 (Horseshoe Island) and *Oceanisphaera marina* UTB 169 (Elephant Point) were the strains with the highest resistance, presenting resistance to the six antibiotics evaluated. The strains of *Psychrobacter glaciei* UTB 167 and *Pseudomonas mandelii* UTB 115, from Deception Island and Livingston Island respectively, showed resistance to four antibiotics (PNC, DLX, AMX and AMP). On the other hand, *Pseudomonas antarctica*, *Janthinobacterium svalbardense* and *Exiguobacterium* sp., isolated from the Byers Peninsula, were mostly sensitive to most antibiotics, except for their resistance to dicloxacillin (Table 3).

TABLE III
ANTIBIOTIC RESISTANCE PROFILES OF BACTERIAL STRAINS ISOLATED FROM DIFFERENT LOCATIONS IN ANTARCTICA

Isolation location	Code	PNC	DLX	AMX	AMP	CEF	SUF
Deception Island	UTB 113	+	+	-	-	-	-
	UTB 145	-	+	-	-	-	-
	UTB 167	+	+	+	+	-	-
Livingston Island	UTB 115	+	+	+	+	-	-
	UTB 117	+	+	-	+	-	-
	UTB 118	-	+	-	-	-	-
Artigas Base	UTB 170	-	+	-	-	-	-
	UTB 171	+	+	+	+	-	-
Horseshoe Island	UTB 173	+	+	+	+	+	+
	UTB 174	+	+	+	+	-	-
Half Moon Island	UTB 160	+	-	-	-	-	-
Elephant Point	UTB 169	+	+	+	+	+	+
	UTB 172	+	+	+	+	+	-
Byers Peninsula	UTB 106	-	+	-	-	-	-
	UTB 109	-	+	-	-	-	-
	UTB 168	-	+	-	-	-	-

On the other hand, the results show that dicloxacillin had the highest resistance rate, with 15 resistant strains (93.75%) and only one susceptible strain (6.25%). Penicillin followed with 62.5% resistance. In contrast, cephalexin and sulfamethazole were the antibiotics with the lowest resistance, with only 12.5% resistant strains and 87.5% susceptible strains. Amoxicillin and ampicillin showed a more balanced distribution, with 43.75% and 50% resistance, respectively, indicating variability in

susceptibility to these antibiotics.

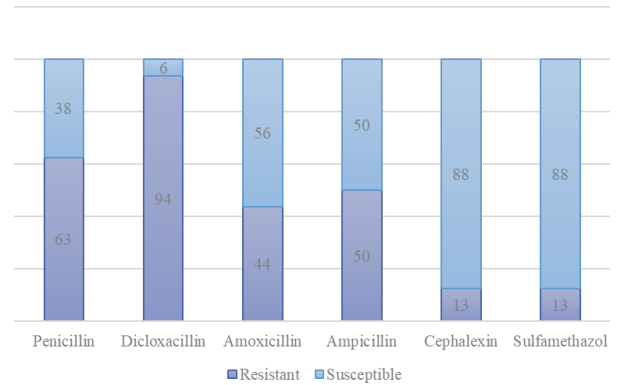


Fig. 2 Distribution of antibiotic resistance and susceptibility in bacterial strains isolated from Antarctica.

The results show that resistance varies according to geographic location and level of human activity. Strains isolated from Livingston Island showed the highest percentage of resistance (78%), followed by those from Deception Island and Byers Peninsula, both with 61% resistance. In contrast, strains from Artigas Base, Herradura Island, Media Luna Island and Punta Elefante showed the lowest levels of resistance (17-25%), with a high percentage of susceptibility (75-83%). Regarding the impact of human activity, sites with high intervention showed 69% resistance,

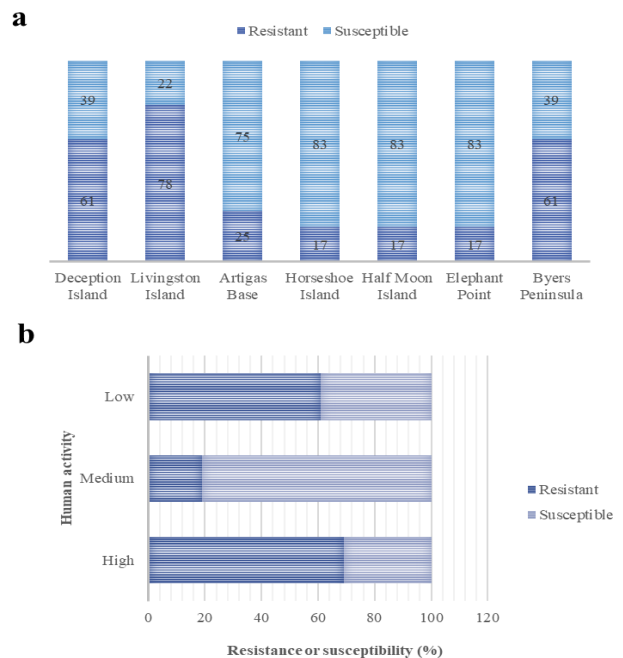


Fig. 3 Distribution of antibiotic resistance and susceptibility in bacterial strains isolated from

while those with medium intervention showed significantly lower resistance (19%). On the other hand, sites with low intervention showed 61% resistance, similar to the values observed on Deception Island and Byers Peninsula.

IV. DISCUSSION

Antibiotics are considered emerging contaminants due to their persistence in the environment and their ability to induce resistance in microbial communities [10]. Their presence in aquatic and terrestrial ecosystems, because of excessive use in medicine and livestock farming, favors the selection of resistant bacteria, which represents a threat to human health by reducing the effectiveness of antimicrobial treatments [19]. In this context, studying antibiotic resistance in Antarctica is crucial, since this continent represents a pristine ecosystem where the detection of resistant bacteria could reveal the global spread of resistance genes, even in remote regions, providing key information to understand the evolution and dissemination of these mechanisms in the environment [20]. Therefore, this study evaluated resistance to different types of antibiotics in strains isolated from Antarctica and its relationship with the level of human intervention at the isolation sites.

The results obtained in this study show variability in the antibiotic resistance profiles between the different bacterial strains isolated in different locations in Antarctica. The presence of antibiotic-resistant bacteria in Antarctica has been previously reported [13] [21-23], suggesting that antimicrobial resistance is not a phenomenon exclusive to environments impacted by human activities. Studies carried out in different regions of the continent have identified strains with intrinsic and acquired resistance, showing the presence of resistance genes, even in ecosystems considered pristine [14] [21]. Among the strains analyzed, *Psychrobacter glaciei* UTB 173 (Horseshoe Island) and *Oceanisphaera marina* UTB 169 (Elephant Point) were the ones that showed the highest resistance, showing simultaneous resistance to the six antibiotics evaluated. On the other hand, *Psychrobacter glaciei* UTB 167 (Deception Island) and *Pseudomonas mandelii* UTB 115 (Livingston Island) showed resistance to four antibiotics (PNC, DLX, AMX and AMP), which reinforces the idea that some bacterial species in Antarctica may have shared resistance mechanisms. The resistance of the species *Psychrobacter glaciei* has been previously reported in the study carried out by [24] where the strain SC80A.2 isolated from a block of the Scarisoara ice cave, related to this species, presented resistance to 7 different classes of antibiotics.

For their part, [25] reported the isolation of strain 6A1 from marine sediments in Fildes Peninsula Bay, King George

Island, Antarctica. This strain, related to the species *Pseudomonas mandelii*, showed multiple resistance to various antibiotics. In addition, antibiotic resistance of the genus *Pseudomonas* has been widely reported, which is probably related to its ability to colonize different habitats due to its metabolic versatility and robustness [26]. In the case of *Oceanisphaera marina*, this is the first report of antibiotic resistance for this species.

Most strains tested were resistant to the beta-lactam antibiotics dicloxacillin and penicillin. Similar results have been previously reported in strains isolated from Antarctica [14] [21-22]. In extreme environments such as Antarctica, antibiotic production represents a competitive advantage to efficiently compete for nutrients that are limited in this type of ecosystem [14]. In addition to ecological factors, resistance to beta-lactam antibiotics may also be related to anthropogenic activity or bird migration [21].

Finally, the results indicate that antibiotic resistance varies according to geographic location and the level of human intervention at isolation sites. Strains isolated from Livingston and Deception Islands showed the highest percentage of resistance. These islands are located in the South Shetland Islands in Antarctica and are home to permanent and temporary research bases of several countries. In addition, tourism activities in Antarctica have increased in recent years [21]. The relationship between the prevalence of antibiotic-resistant genes and anthropogenic activity has been previously reported [21-22] [27], since the treatment of wastewater generated at research bases is insufficient to eliminate this type of compounds [13]. In addition, it has also been reported that microplastics are potential vectors of antibiotic-resistant genes [14]. In the case of the Byers Peninsula, which is an area of low anthropogenic activity since it is a specially protected area, it was shown that the bacteria isolated from this area showed a high resistance to different antibiotics. These results may suggest natural mechanisms, such as the presence of resistance genes in native microbiomes or adaptation to extreme conditions. Some studies have shown that extreme temperature, pH and salinity can induce phenotypic and genotypic adaptations in bacteria [21]. In addition, most of the antibiotics used in clinics originate from producer microorganisms, which release these substances in their natural environment, exposing other species and favoring the evolution of resistance mechanisms both in the producer organisms and in those that cohabit with them [28]. Likewise, some resistance genes present in the environment could confer protection against antibiotics for clinical use that have structural or chemical similarities with natural toxic compounds [23].

V. CONCLUSION

This study demonstrates that antibiotic resistance in bacteria isolated from Antarctica varies by geographic location and level of human intervention at isolation sites. The high resistance observed in strains of *Psychrobacter glaciei* and *Oceanisphaera marina*, and in areas of low human intervention such as the Byers Peninsula, suggests that both natural and anthropogenic factors may be influencing the spread of resistance genes in this extreme ecosystem. Furthermore, the relationship between the prevalence of resistance genes and human activity, particularly on islands with growing research and tourism bases, reinforces the hypothesis that wastewater discharges and other anthropogenic impacts could contribute to the spread of antimicrobial resistance in these polar regions. These findings demonstrate the importance of continuing studies on antimicrobial resistance in Antarctica, not only to understand the dynamics of these mechanisms in pristine ecosystems, but also to assess their impact on the global spread of antibiotic resistance.

ACKNOWLEDGMENT

This work was supported by a grant from Universidad Tecnológica de Bolívar. Logistical support for displacement and sampling at the different points evaluated in Antarctica was provided by the Colombian Ocean Commission (CCO) coordinator of the Colombian Antarctic Program - PAC, Spanish Polar Committee - CPE, the Spain Navy, and the BIO Hespérides Oceanographic Research Vessel (A-33).

REFERENCES

- [1] B. J. Werth, "Generalidades sobre los fármacos antibacterianos," Manual MSD Versión Para Profesionales, Nov. 15, 2023. <https://www.msmanuals.com/es/profesional/enfermedades-infecciosas/bacterias-y-f%C3%A1rmacos-antibacterianos/generalidades-sobre-los-f%C3%A1rmacos-antibacterianos>
- [2] R.I. Aminov, R.I. Mackie, Evolution and ecology of antibiotic resistance genes, *FEMS Microbiol. Lett.* 271:147–161. 2007
- [3] R. Aminov. (2017). History of antimicrobial drug discovery: Major classes and health impact. *Biochemical pharmacology*, 133, 4-19.
- [4] A. Koul, N. Dendouga, K. Vergauwen, B. Molenberghs, L. Vranckx, R. Willebrords, Z. Ristic, H. Lill, I. Dorange, J. Guillemont, D. Bald, K. Andries, Diarylquinolines target subunit c of mycobacterial ATP synthase, *Nat. Chem. Biol.* 3 (6) (2007) 323–324, <http://dx.doi.org/10.1038/nchembio884>.
- [5] N. Vázquez-Laslop, & A. S. Mankin. How macrolide antibiotics work. *Trends in biochemical sciences*, 43(9), 668-684. 2018.
- [6] W. Baran, E. Adamek, J. Ziemiańska & A. Sobczak. Effects of the presence of sulfonamides in the environment and their influence on human health. *Journal of hazardous materials*, 196, 1-15. 2011.
- [7] T. Heberer. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicology Letters*, 131(1–2). 2002. [https://doi.org/10.1016/S0378-4274\(02\)00041-3](https://doi.org/10.1016/S0378-4274(02)00041-3)
- [8] N. Kemper. Veterinary antibiotics in the aquatic and terrestrial environment. *Ecological Indicators*, 8(1). 2008. <https://doi.org/10.1016/j.ecolind.2007.06.002>
- [9] B. Li, & T. Zhang. Biodegradation and adsorption of antibiotics in the activated sludge process. *Environmental science & technology*, 44(9), 3468-3473. 2010.
- [10] I. T. Carvalho & L. Santos. Antibiotics in the aquatic environments: a review of the European scenario. *Environment international*, 94, 736-757. 2016.
- [11] A. Kumar & D. Pal. Antibiotic resistance and wastewater: correlation, impact and critical human health challenges. *Journal of environmental chemical engineering*, 6(1), 52-58. 2018.
- [12] J. Davies & D. Davies. Origins and evolution of antibiotic resistance. *Microbiology and molecular biology reviews*, 74(3), 417-433. 2010.
- [13] F. E. L. X. Hernández, N. Calisto-Ulloa, C. Gómez-Fuentes, Gómez, J. Ferrer, G. González-Rocha, ... & M. Montory. Occurrence of antibiotics and bacterial resistance in wastewater and sea water from the Antarctic. *Journal of hazardous materials*, 363, 447-456. 2019.
- [14] K. Hwengwere, H. Paramel Nair, K. A. Hughes, L. S. Peck, M. S. Clark, & C. A. Walker. Antimicrobial resistance in Antarctica: is it still a pristine environment?. *Microbiome*, 10(1), 71. 2022.
- [15] J. Hernández & D. González-Acuña. Anthropogenic antibiotic resistance genes mobilization to the polar regions. *Infection ecology & epidemiology*, 6(1), 32112. 2016.
- [16] C. Rubiano-Labrador, R. Acevedo-Barrios, A. García Lazaro, L. Ward Bowie, A. K. Támara Acosta & B. Mercado Molina. *Pseudomonas* strains from the Livingston Island, Antarctica: a source of cold-active hydrolytic enzymes. 2022.
- [17] Y. Ledesma & Y. Barreto. Aislamiento de bacterias degradadoras de Tereftalato de Polietileno (PET) provenientes de sedimentos marinos de la Antártida. Thesis. Programa Ingeniería Química. Universidad Tecnológica de Bolívar. 2023
- [18] R. L. Alcántara, V. M. Padilla, E. G. Lazo, R. M. Castillo, Y. M. López, & M. C. Bacab. Aislamiento y evaluación de la actividad antimicrobiana del extracto crudo de la bacteria marina *Pseudoalteromonas* sp. *Revista Mexicana de Ciencias Farmacéuticas*, 43(4), 38-46. 2012.
- [19] Scott, L. C., Lee, N., & Aw, T. G. (2020). Antibiotic resistance in minimally human-impacted environments. *International journal of environmental research and public health*, 17(11), 3939.
- [20] T. Segawa, A. Takahashi, N. Kokubun & S. Ishii. Spread of antibiotic resistance genes to Antarctica by migratory birds. *Science of the Total Environment*, 923, 171345. 2024.
- [21] J. Depta, & P. Niedźwiedzka-Rystwej. The phenomenon of antibiotic resistance in the polar regions: an overview of the global problem. *Infection and drug resistance*, 1979-1995. 2023.
- [22] D. Jara, H. Bello-Toledo, M. Domínguez, C. Cigarroa, P. Fernández, L. Vergara, ... & G. González-Rocha. Antibiotic resistance in bacterial isolates from freshwater samples in Fildes Peninsula, King George Island, Antarctica. *Scientific reports*, 10(1), 3145. 2020.
- [23] A. E. Marcoleta, P. Arros, M. A. Varas, J. Costa, J. Rojas-Salgado, C. Berrios-Pastén, ... & R. Lagos. The highly diverse Antarctic Peninsula soil microbiota as a source of novel resistance genes. *Science of the Total Environment*, 810, 152003. 2022.
- [24] V. I. Paun, P. Lavin, M. C. Chifiriuc, & C. Purcarea. First report on antibiotic resistance and antimicrobial activity of bacterial isolates from 13,000-year old cave ice core. *Scientific reports*, 11(1), 514. 2021.

- [25] S. Higuera-Llantén, F. Vásquez-Ponce, M. Núñez-Gallegos, M. S. Pavlov, S. Marshall & J. Olivares-Pacheco. Phenotypic and genotypic characterization of a novel multi-antibiotic-resistant, alginate hyperproducing strain of *Pseudomonas mandelii* isolated in Antarctica. *Polar Biology*, 41, 469-480. 2018.
- [26] P. Laborda, S. Hernando-Amado, J. L. Martínez, & F. Sanz-García. Antibiotic resistance in Pseudomonas. In *Pseudomonas aeruginosa: Biology, Pathogenesis and Control Strategies* (pp. 117-143). Cham: Springer International Publishing, 2022.
- [27] G. Na, W. Zhang, H. Gao, C. Wang, R. Li, F. Zhao, ... & C. Hou. (2021). Occurrence and antibacterial resistance of culturable antibiotic-resistant bacteria in the Fildes Peninsula, Antarctica. *Marine Pollution Bulletin*, 162, 111829.
- [28] V. M. D'Costa, K. M. McGrann, D. W. Hughes, G. D. Wright. Sampling the antibiotic resistome. *Science* (80-) 311, 374-377. <https://doi.org/10.1126/science.1120800>. 2006.