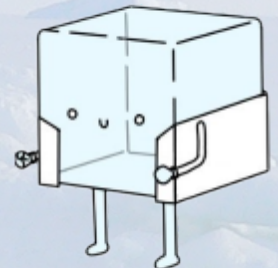




TAN TÁRI BRICKS

DESIGN ROADMAP

OUR WAY TO IDL



PROBLEM IDENTIFICATION

Antibiotics are drugs based on killing the microorganisms that cause diseases. Although they are generally effective, their use has the problem that microorganisms capable of resisting these antibiotics are selected during the treatment with these drugs. Antibiotic resistance has quickly spread among pathogen bacteria since the beginning of its use and today this phenomenon is considered a threat to global health. WHO projected that by 2050 infections caused by pathogen resistant to antibiotics will be responsible for 10 million deaths per year. As a result, it is recognized that to address this problem, an interdisciplinary approach is required to establish policies and actions that elucidate the causes and scopes of this emergency in different ecosystems.

Developing countries are most affected by this emergency. The lack of education generates a lack of awareness in the population about the correct use of antibiotics, and this is maximized by the lack or absence of legislation that regulates the use of antibiotics to prevent its misuse, so antibiotic resistance does not spread or it spreads more slowly.

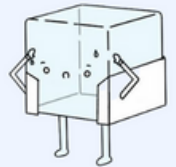
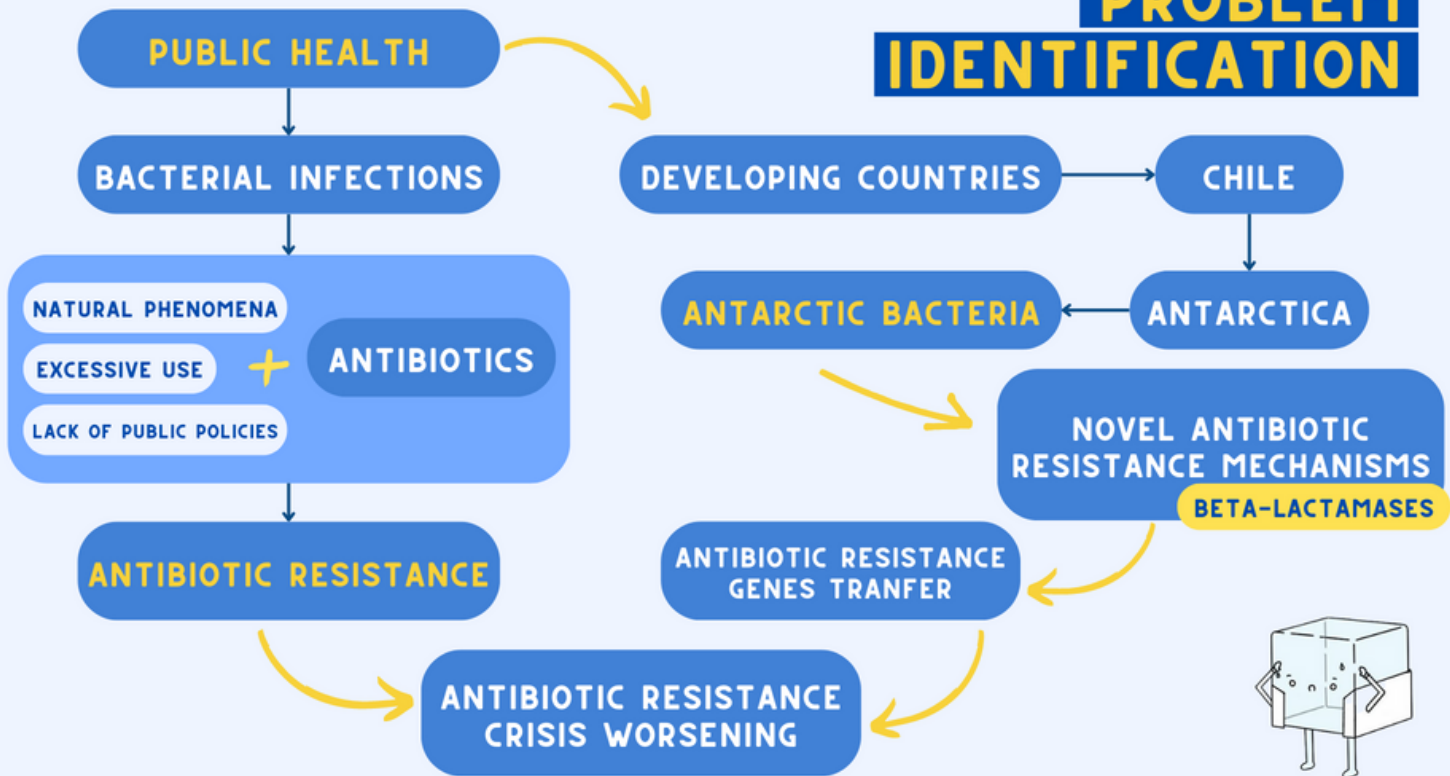
In Chile, our team's home country, we have identified an additional problem (image below). Antarctica, the southernmost continent in the world, harbors a particular ecosystem. Many unique bacteria have been identified in this ecosystem, some of them showing high levels of resistance to various antibiotics. Therefore, this ecosystem harbors an extensive repertoire of putative resistance genes coding an unexplored variety of antibiotic-inactivating enzymes.

To this is added that the characteristics of Antarctica have made it a constant focus of interest, which means that there is an increasing transit of people between Chile and Antarctica, as Chile is the main entrance to Antarctica. This increases the number of microorganisms that could be transmitted to Chile from Antarctica and, therefore, increases the probability of introducing antibiotic-resistant microorganisms and antibiotic-resistance genes.

For this reason, the soils of the Antarctic Peninsula and the microorganisms that inhabit them should be monitored as a potential source of new resistance mechanisms since they constitute a repertoire of unexplored genes that can allow us to understand better and combat antibiotic resistance. At Tan Tári Bricks, we recognize that these new resistance mechanisms must be urgently studied due to climate change significantly affecting Antarctica, generating an increase in its temperatures and constant melting. As the only Chilean team, we feel responsible for taking this knowledge from the southernmost part of the world and taking it to actions that contribute to diminishing the spread of antibiotic resistance.

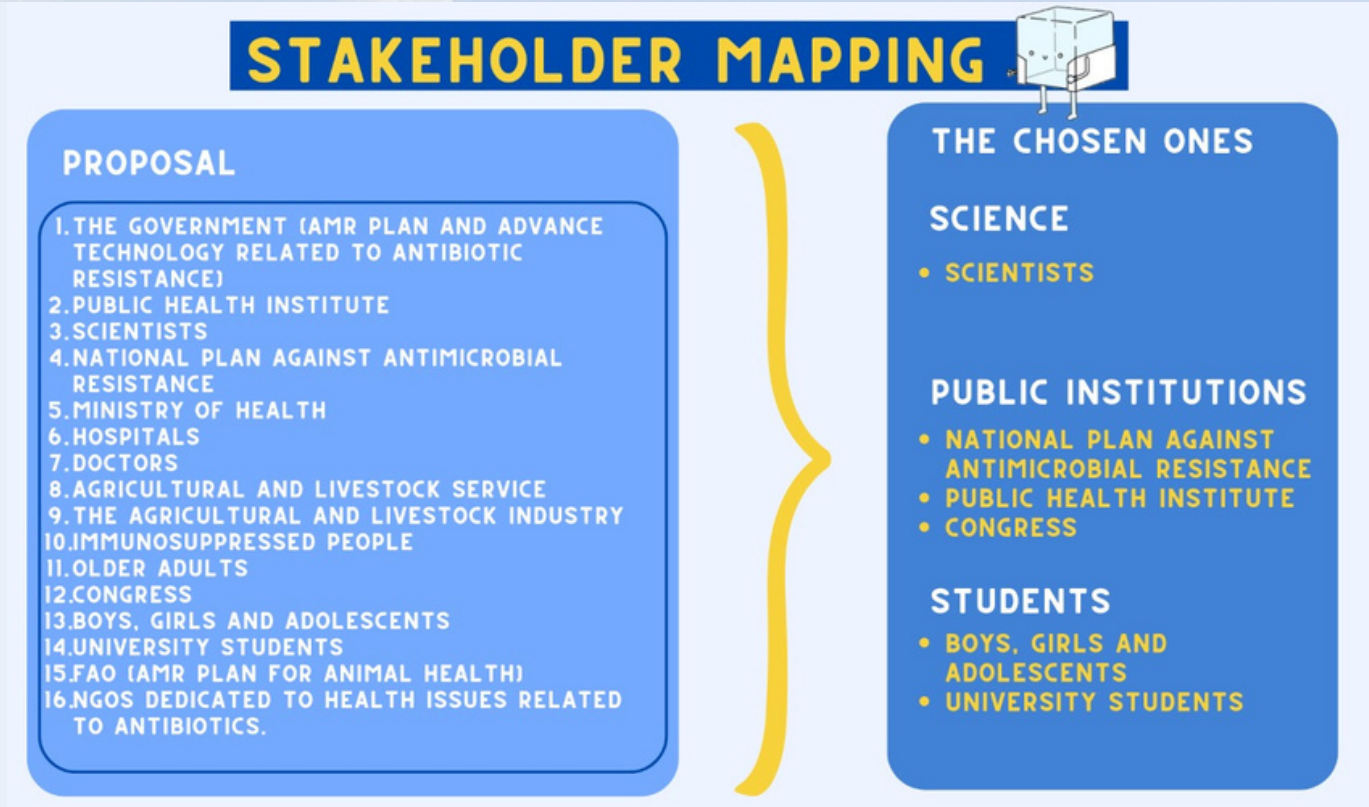
PROBLEM IDENTIFICATION

PROBLEM IDENTIFICATION



STAKEHOLDERS MAPPING

To design a solution for the previously identified problem, we defined the actors involved. For this first step, we went into a stakeholders mapping process (figure below). Considering that this problem affects the whole society, we choose six main stakeholders and divided them per area: science, public institutions, and students.



SOLUTION: DESIGN THINKING METHODOLOGY

Then, to create the solution, we proceeded to use the Design Thinking Methodology which consists of 5 iterative steps: Empathize, Define, Ideate, Prototype, and Test, but we only considered the first three, since iDL goes as far as the solution design.

EMPATHIZE

DEFINE

IDEATE

EMPATHIZING PHASE

Firstly, we conducted intensive research about how the antibiotic resistance problem affects our country and how our stakeholders are involved.

1. Thanks to literature and conversations with our mentors -who had previous investigations on this topic- we realized that **beta-lactamase synthesis is one of the most common ways to develop antibiotic resistance.**
2. Thanks to literature, we detected that these novel antibiotic resistance mechanisms have as one of their causes the irresponsible use of antibiotics by the population, so we made the obvious question why? **We talked with biology school teachers, as one of our areas of stakeholders is students.** They told us that **antibiotic resistance** was recently included in the national school curriculum, addressed in the unit on microorganisms, but it is not a learning objective. Also, we researched about **public campaigns about responsible antibiotic use** and found that there are not enough public campaigns on this topic; the first and last one were in 2019.
3. On the other hand, we detected another cause of the novel antibiotic resistance mechanisms: the lack of national surveillance and national regulation in livestock and fish farming. Again, we asked ourselves why?, so for the lack of surveillance we decided to **contact our stakeholders from the public institutions area**, and we asked them why we were not present in the Global Antimicrobial Resistance and Use Surveillance System (GLASS). In the absence of livestock and fish farming regulation, **we followed the law processing of the labeling antibiotics law.**

DEFINITION PHASE

For the definition step, we consider the complete information we collected and defined three lines of action:

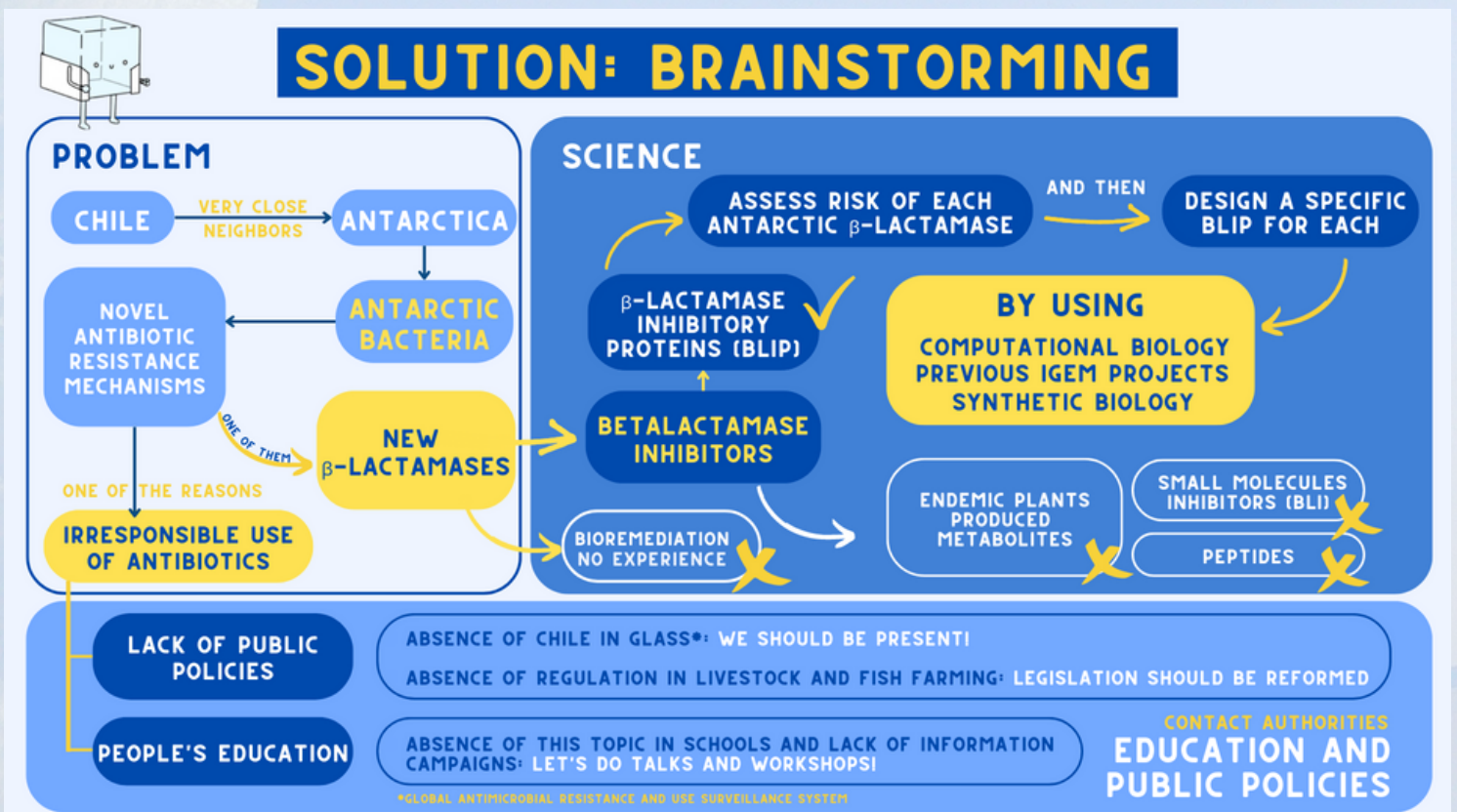
- 1) Science with a focus on beta-lactamases.
- 2) People's education focused on raising awareness about the use of antibiotics.
- 3) Public policies with the focus on helping the integration of Chile to GLASS and the law regarding the labeling of antibiotics.

IDEATION PHASE: BRAINSTORMING

In the ideation phase, we made a brainstorming for each line of action: for the first one, Science with the focus on betalactamases, many solutions were proposed and several ideas were discarded, finally leaving the production of betalactamase inhibitor proteins (more information in the Methodology section). This was perfected over time as we investigated more about it in the literature, and had our Tan Tári Team Academy sessions (more information in the Methodology section). Also, it should be noted that a watershed was a protein talk with a national biotech startup, Protera, which led us to decide to use protein. Finally, we contacted the Public Health Institute (ISP in Spanish), looking for their opinion about our project. So the ideation process was an iterative one, where it was constantly improving.

For the second one, People's education focused on raising awareness about the use of antibiotics, ideas like science fairs, educational games and video games were proposed. However, the winner was workshops for school students in our University laboratories, a section on this topic in an open symposium for school and university students and educational posts on our social networks.

Finally, for the third one, Public policies with the focus on GLASS and the promotion of the labeling of antibiotics law, we discussed different alternatives, but two were chosen: 1) A report on Chilean legislation on resistance and 2) A letter addressed to the appropriate person in Congress highlighting the problem and giving suggestions for action, including the incorporation of Chile to GLASS (after having discussed with the Chilean ISP - Chilean Public Health Institute)



METHODOLOGY

The methodology was a result of many sessions of discussion. We created a space called Tan Tári Team Academy, where team members presented scientific articles related to different parts of the solution to mentors and instructors looking for feedback. Also, we invited professors to this space to teach us about areas in which we had no expertise. We want to highlight the classes that Dr. Carlos F. Lagos (USS, Chile) gave us in this space. He is a pharmaceutical chemist and his vast knowledge in protein structure bioinformatics and his feedback were crucial for us. Thus, thanks to this space and previous team knowledge, we could design a proper methodology to develop our solution iteratively.

COMPUTATIONAL BIOLOGY PIPELINE

- Antarctic Beta-Lactamases selection
 - In silico beta-lactamases prospection in Chilean Antarctic soil metagenomes
 - Functional classification of antarctic beta-lactamases candidates.
 - Filtering and selection of two carbapenemases candidates: Class A BKC-beta-lactamase and Class D OXA-beta-lactamase.
- Structural Modeling of selected beta-lactamases
 - Structural Modeling and Molecular Dynamic refinement of selected beta-lactamases.
- Beta-Lactamase - Beta-Lactamase Inhibitor Protein complex structural modeling
 - Molecular Docking of selected beta-lactamases and BLIP-I protein complexes.
- In silico optimization of BLIP-I
 - *In silico* molecular affinity optimization of BLA - BLIP-I complex interaction by site specific mutagenesis of canonical BLIP-I.

EXPERIMENTAL WORKFLOW

- Antarctic beta-lactamases activity spectrum characterization.
 - Measurement of enzymatic activity of beta-lactamases and their specificity for different beta-lactam antibiotics using *E.coli* as host.
- Activity testing of in silico optimized BLIP-I structural variants
 - Development of synthetic biology-based screening platform in *E.coli* to test the effect of different beta-lactamase inhibitor protein BLIP-I on class A and D beta-lactamases.
- Bulk production of beta-lactamase inhibitor protein BLIP-I.

The BLIP-I structural variant with the highest and most efficient inhibition activity will be optimized for bulk production in *E. coli*.

METHODOLOGY

METHODOLOGY

COMPUTATIONAL BIOLOGY PIPELINE

ANTARCTIC BLA
METAGENOMIC
PROSPECTION

BLA STRUCTURAL
MODELLING AND
CHARACTERIZATION

BLA-BLIP DOCKING AND
COMPLEX MODELLING

RATIONAL DESIGN OF
BLIP VARIANTS WITH
↑ BINDING AFFINITY

EXPERIMENTAL WORKFLOW

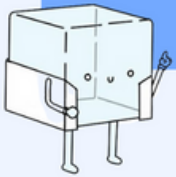
ANTARCTIC BLA
ACTIVITY SPECTRUM
CHARACTERIZATION

BLA ACTIVITY INHIBITION ASSAYS WITH
OPTIMIZED BLIPS

PRODUCTION OF BEST
BLIPS FUSED TO AN
UPTAKE DOMAIN

BLA: BETA-LACTAMASE

BLIP: BETA-LACTAMASE INHIBITOR PROTEIN

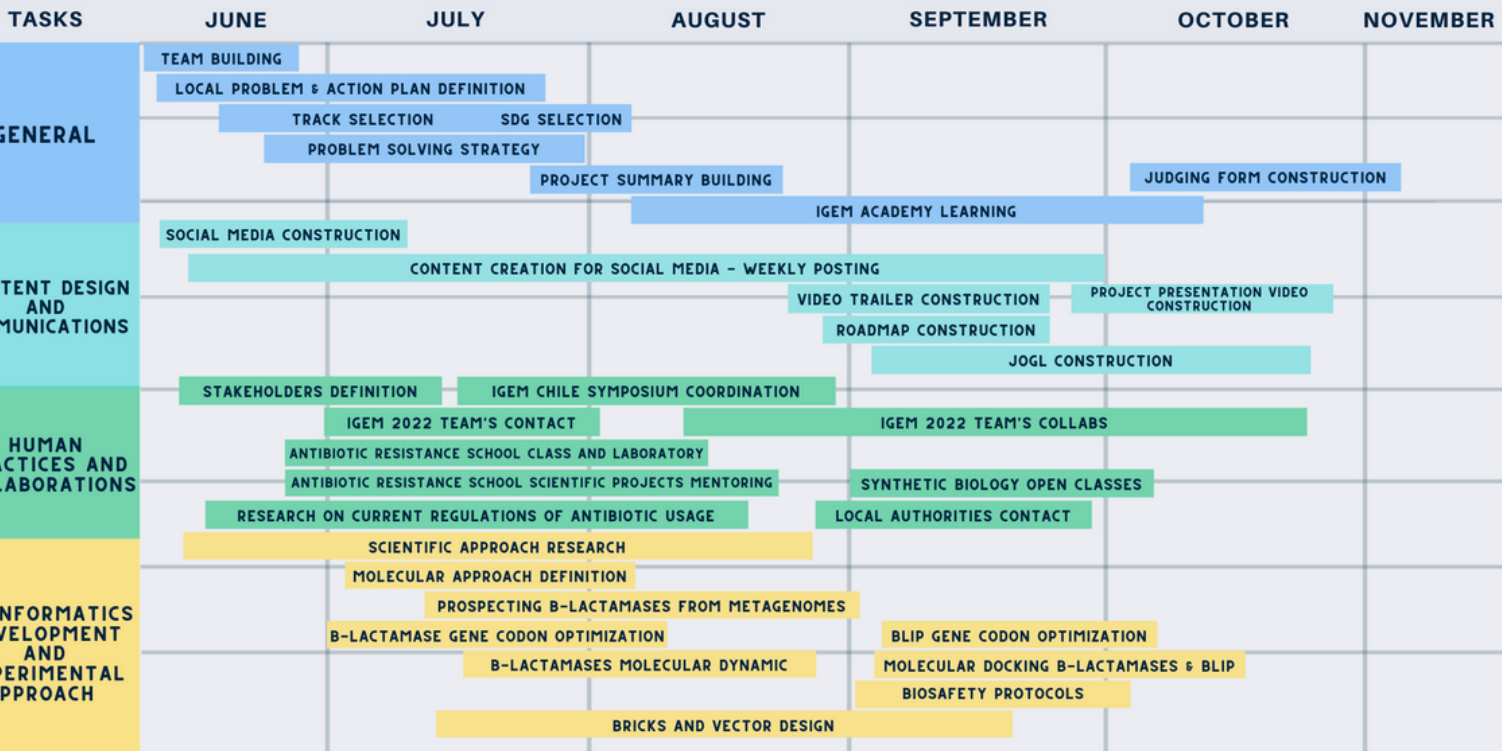


TIMELINE

In order to reach our goals, we created a Gantt Chart -which is reflected in the timeline below- with the different activities deadlines per commission. Thus, each team member could organize their time to comply with them and be aware of the activities of each area.

PROJECT TIMELINE

TAN TÁRI BRICKS





BONUS: THE STORY BEHIND OUR TARITO

Chile, known as the end of the world, has always been a country that has been explored countless times throughout history. The eagerness for the discovery of its inhabitants has led them to explore it since pre-Columbian times, and visitors from other continents have even explored it. Despite this, not everything has been discovered, and we continue to explore these southern lands to find local answers to global problems. So, this ancestral tradition of exploring the south is maintained to this day, the eagerness for exploration and discovery has not changed, but the tools and the problems have. We continue to explore the south to survive, and the solution lies in the coldest of the lands: Tan Tári.

TARITO "EL HIELITO"

