UNIVERSIDAD AUTONOMA DE CHILE

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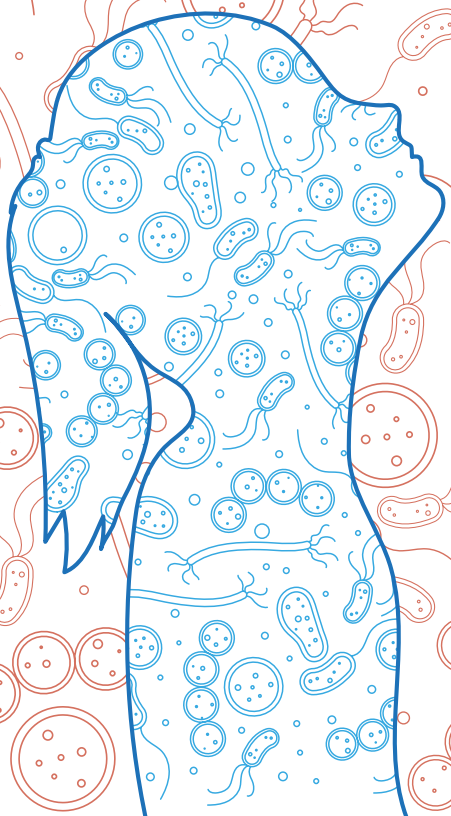
BACTERIA

WHY DO THEY MAKE ME SICK?

Dr. Gino Corsini Acuña



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Universidad Autónoma de Chile

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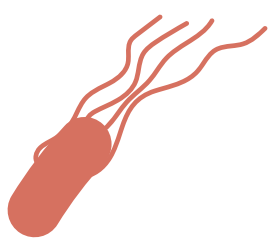
Through digital communication and the development of outreach, editorial products, the intent is to publicize high-impact research carried out in the country and to promote the scientific culture.



Dr. Gino Corsini started out in science through his university studies after graduating as a Biochemist from Universidad de Chile, the institution where he obtained his PhD in Science, with a specialization in Microbiology.

He has worked as a professor teaching Biochemistry, Microbiology and Molecular Biology for undergraduate and graduate students.

He has actively participated as a member of the board of the Chilean Society for Microbiology, Chilean Society of Biochemistry and Molecular Biology (SBBMCH, for its acronym in Spanish), the Chilean Society of Biology (SBCH, for its acronym in Spanish) and the Latin American Association of Microbiology (ALAM, for its acronym in Spanish). At present, he holds the position of Director of the Institute of Biomedical Science at Universidad Autónoma de Chile.

BACTERIA

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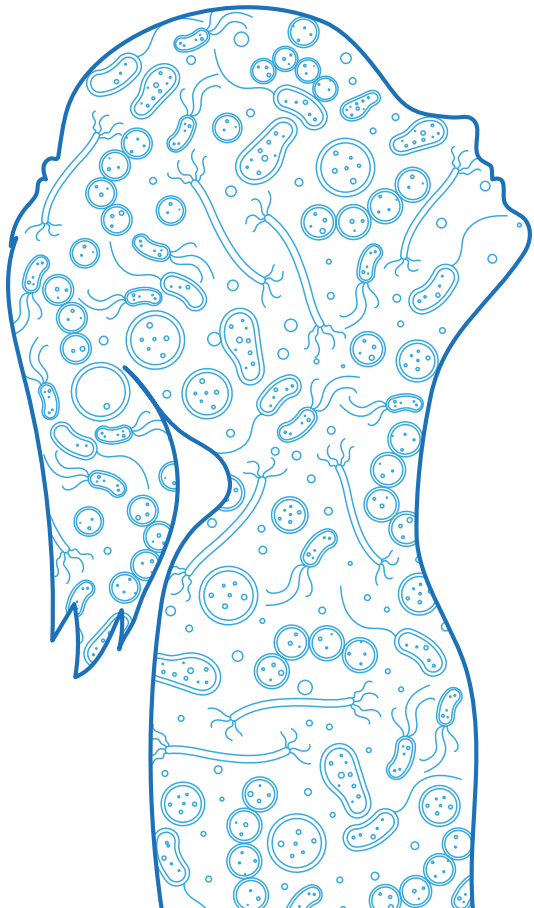
Dr. Gino Corsini Acuña



Bacteria, why do they make me sick?
Dr. Gino Corsini

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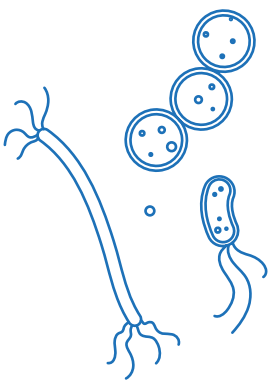


CHAPTER 1

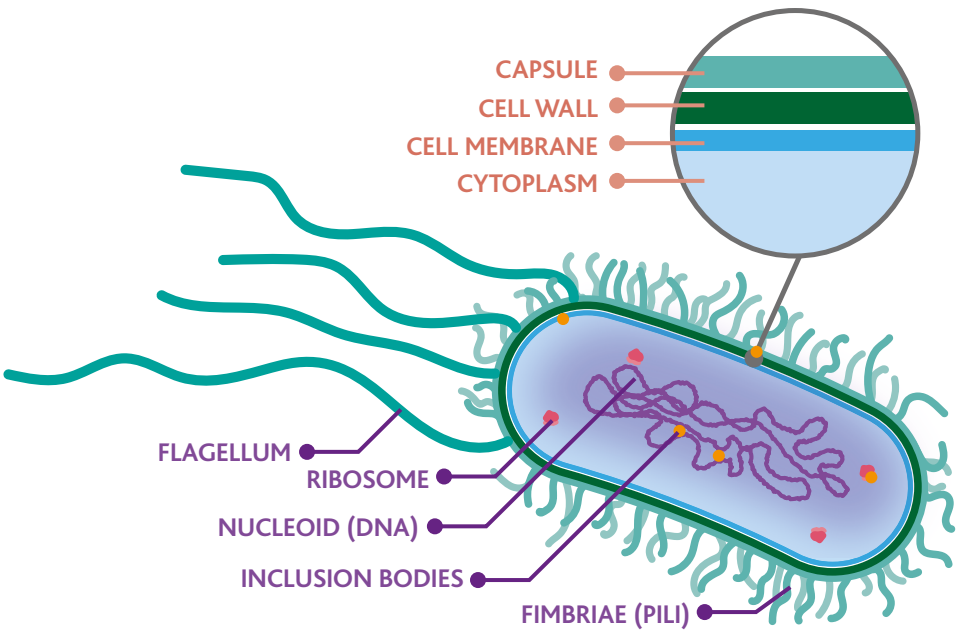
WHAT ARE BACTERIA?

Before starting,
How would you define a bacterium?
What bacteria do you know?

What are bacteria?

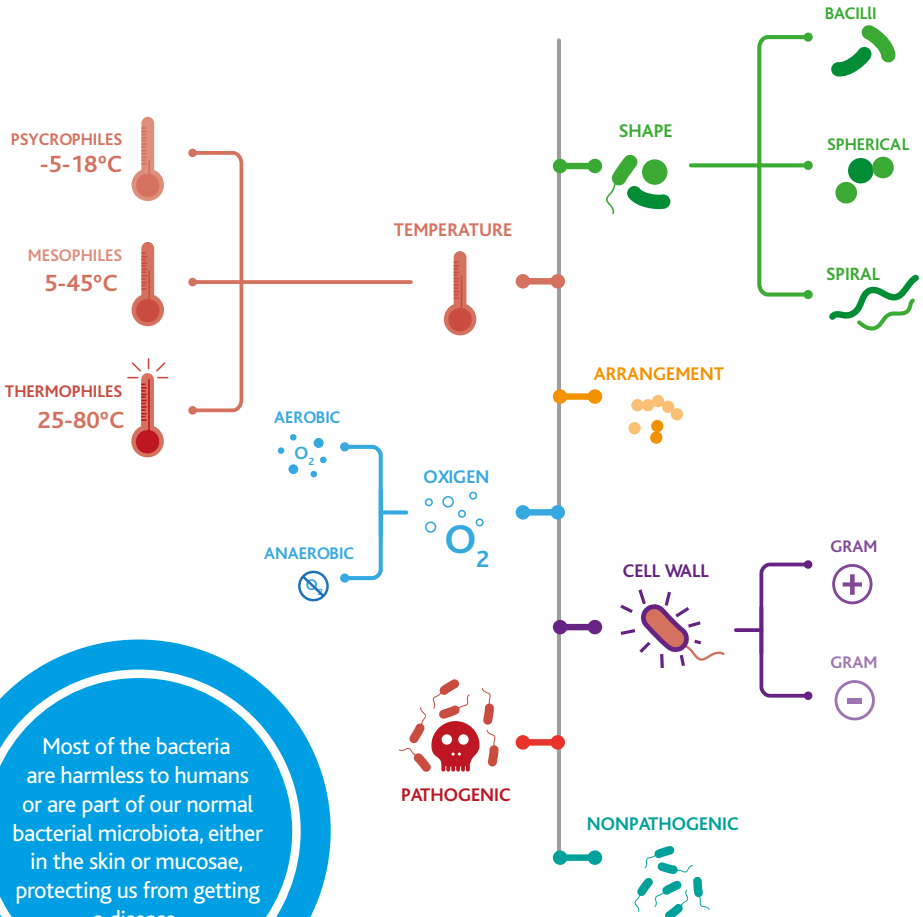
Bacteria are microscopic organisms that lack a defined or structured nucleus. These organisms are classified as prokaryotes.

Structure of a bacterium



Bacterial cells range in size from 0.5 to 5 µm (1 µm is 0.0001 cm). It has a cytoplasm enclosed by a plasma membrane. The outermost layer is the cell wall that varies depending on the type of bacteria. The bacterial genome consists of a long DNA filament, circular and closed, called nucleoid, which is compacted in the cytoplasm and lacks a membrane. The cytoplasm also contains many ribosomes, and in some cases, inclusion bodies. Some bacterial cells also have a capsule, flagellum and fimbriae (also called pili).

There are different criteria to classify bacteria.



Most of the bacteria are harmless to humans or are part of our normal bacterial microbiota, either in the skin or mucosae, protecting us from getting a disease.

Bacteria are classified using different criteria. We can classify them in pathogenic and nonpathogenic; we can also distinguish them based on its morphology, its type of arrangement and also based on its optimal temperature of growth, cell wall and how they respond to oxygen levels.

Can all bacteria make me sick?

The diversity of bacteria is astonishing. In fact, it is believed that the biodiversity of organisms known today, it is only a small percentage of the totality of microorganisms that have not been discovered yet. However, the diversity known today, can be divided into two main groups"

1. **Beneficial bacteria.**
2. **Pathogenic bacterias.**

Beneficial bacteria

MICROBIOTA

Human beings are the habitat of a wide variety of microorganisms named native microbiota. These microorganisms are not only harmless to the host, but they also protect them against pathogenic bacteria, fungi, and viruses that compete for the same available space and nutrients.

Microbiota composition depends on different factors, among which we can highlight the following:

- Personal hygiene
- Diet
- Hydration level
- Drugs use (especially antibiotics)
- Exposure to environmental toxins.

The digestive tract is an example of the importance of the microbiota because, without it, it would be impossible to have a healthy digestive system, due to its contribution to metabolize bile acids and to synthesize vitamins.

In this way, drinking purified or chlorinated water, having a diet with a high or low content of fiber, sugar or fat, may select different intestinal bacteria due to their capacity to use mineral and essential nutrients.

MICROBIOME

The microbiome is defined as the microorganisms located normally in different parts of the body of multicellular organisms, such as the human body.

In this way, the normal microbiome characterizes health, and its alterations may indicate there is a disease. These changes may be harmless when the microbiome and its basic functional properties remain present, but when these functions are lost, diseases may appear.

The information obtained from the study of the different microbiome has added a new concept of disease, one that is caused by a community of microorganisms and not by a particular pathogen. This new definition goes beyond the traditional infectious diseases and it may include immune and metabolic disorders, such as inflammatory bowel disease, obesity, type 2 diabetes, and celiac disease.

Most of the bacteria that colonize the bowel belong to the phylum Actinobacteria, Bacteroidetes and Firmicutes. To establish their niche, some bacteria use antibacterial peptides, bacteriocins or metabolite that prevents the proliferation of other competitive species. These molecules are also beneficial to the host because they eliminate invasive bacteria such as Salmonella spp., Shigella spp., Clostridium difficile, Bacillus cereus, and other pathogens.

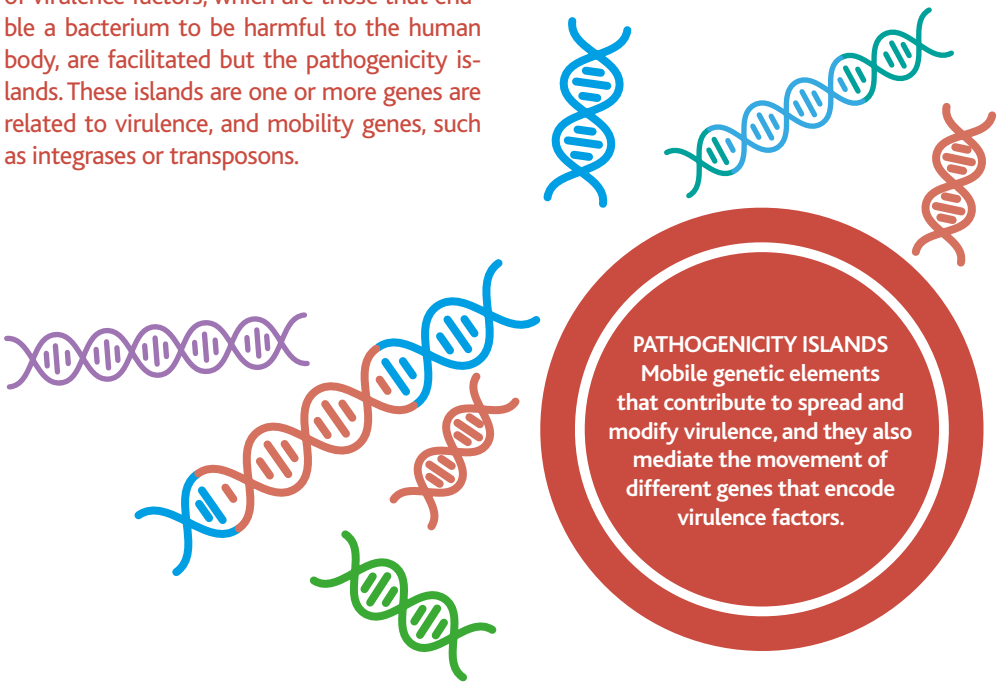
Pathogenic bacteria

Pathogenic bacteria are those that can cause infections in humans or animals and have different virulence factors, which are:

Proteins that contribute to the virulence of the bacteria over the eukaryotic cell, which enables infection.

Interaction mechanisms with mammalian cells.

Currently, it is known that evolution and spread of virulence factors, which are those that enable a bacterium to be harmful to the human body, are facilitated but the pathogenicity islands. These islands are one or more genes are related to virulence, and mobility genes, such as integrases or transposons.

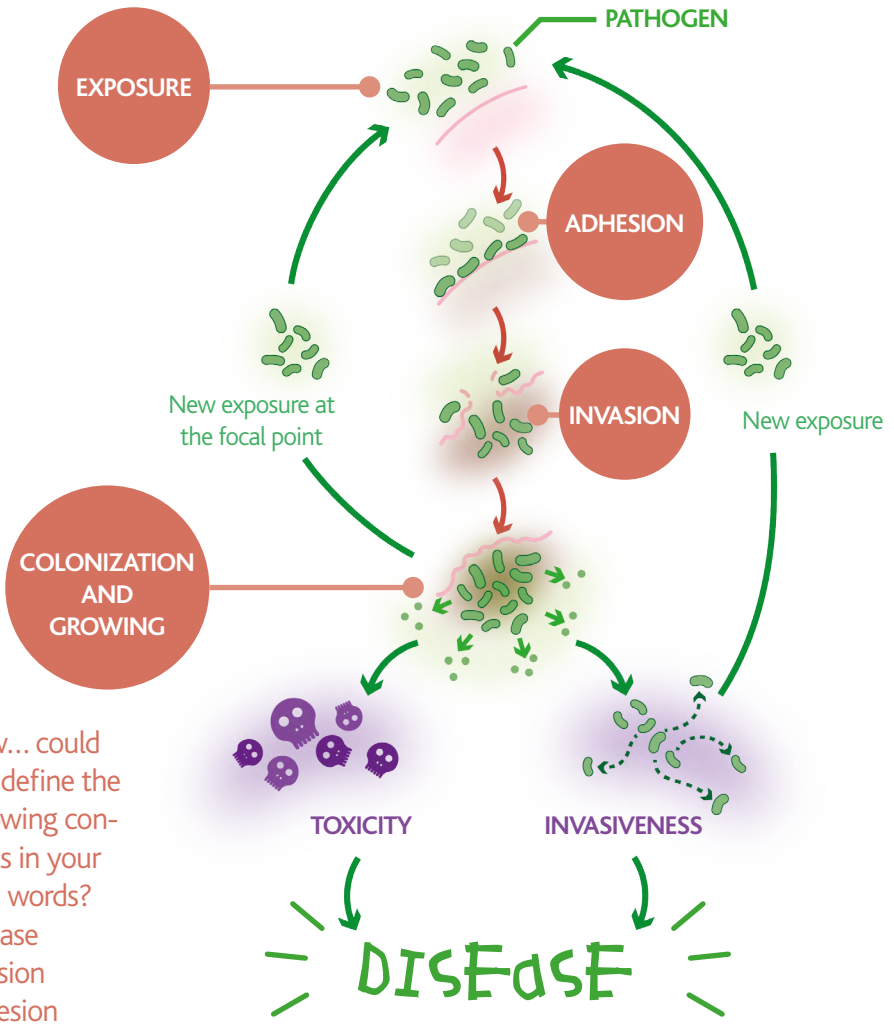


CHAPTER 2

BACTERIA MAKE ME SICK

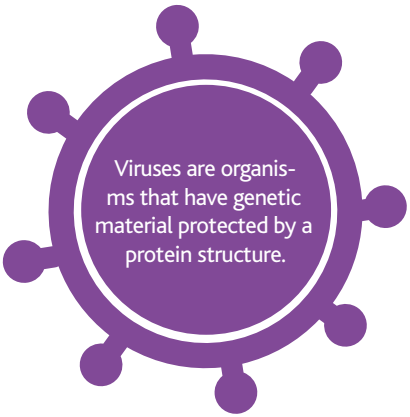
Bacteria and Diseases

A disease is the presence of infection and its symptoms.
The infectious disease, or infective process caused by the virulent bacteria, starts with the colonization, which is the presence of the bacteria in the body.
Then, the infection occurs, which is when bacteria attack the cells or tissues using their mechanisms or virulence factors.



Now... could you define the following concepts in your own words?
Disease
Invasion
Adhesion

Other Pathogenic Microorganisms



The virus

This microscopic organism can cause different diseases when it goes inside a cell and reproduces himself in it.

How does my body react?

However, Infection does not necessarily lead to disease. If the host's immune system acts appropriately, the person can be infected not having the symptoms. Additionally, bacteria are not the only microorganisms able to make human beings sick. There are also different pathogen viruses, such as flu viruses.

Bacteria and body's defenses

As mentioned, a bacterial infection doesn't always lead to disease and this is thanks to our immune system, which, roughly, is able to distinguish self from nonself.

There are two types of immunity: innate or natural immunity (IIS) and the acquired immunity (AIS).

Innate or natural immunity (IIS)

• It's the first line of defense against invasive microorganisms (bacteria, viruses, and fungi).

• It is present since birth; it is nonspecific and lacks memory.

The IIS has three components:

• Physical and chemical barriers; such as skin, cilia, mucosae and secretions. By continuous cleaning, they protect us and prevent the foreign particles entering the body.

• The humoral component is composed of antibodies and the complement system.

• The cellular component, which is the cells that are part of the Innate Immune System: neutrophils, eosinophils, basophils, mast cells, and Natural Killers lymphocytes (NK lymphocytes).

Acquired Immunity (AIS)

• Acquired immunity is not present at birth, it increases with age and it is specific, and it has a memory. This is why it is also called adaptative.

• It is composed of antibodies, T lymphocyte receptors and the molecules of the major histocompatibility complex.

In general, the innate and acquired immune responses are not activated independently. Their optimal performance is when they complement each other.

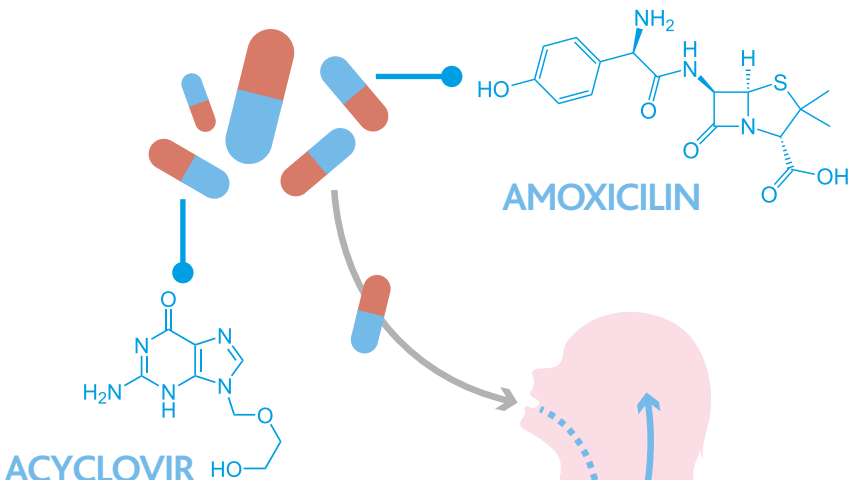
In particular, the AIS' antibodies are able to direct the IIS' components toward the relevant objects.

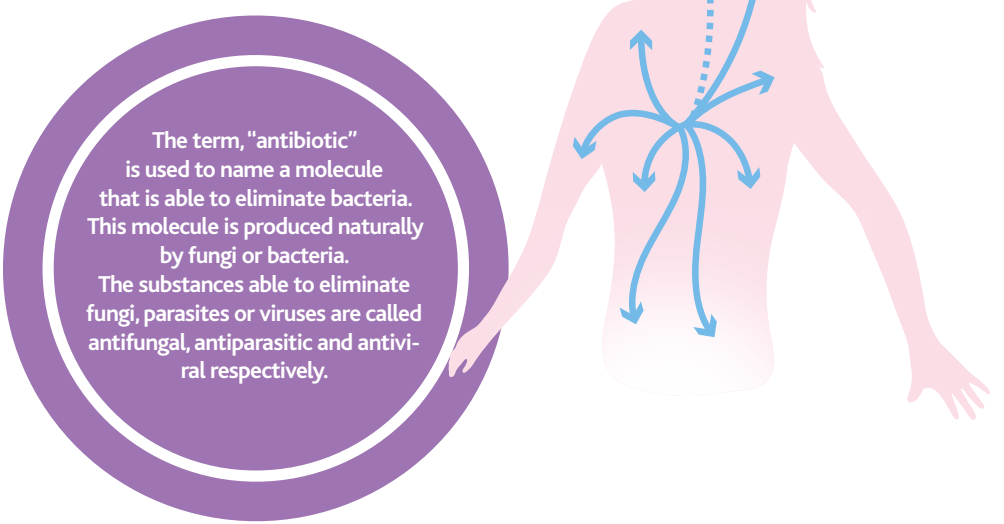
CHAPTER 3

TREATMENT, PREVENCTION AND PERSONAL HYGIENE

Do diseases caused by bacteria have a cure?

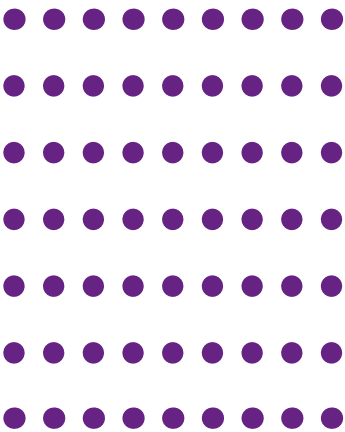
There are different drugs to treat diseases. To treat bacterial diseases, antibiotics are used, such as amoxicillin. To treat diseases caused by viruses, antivirals are used, such as acyclovir.





WHAT IS ACETAMINOPHEN USED FOR?

Over-the-counter painkillers, such as acetaminophen or ibuprofen, moderate the symptoms caused by bacterial diseases, but they do not cure the disease. Therefore it is important a timely medical diagnosis and the appropriate treatment for the specific bacterium causing the infection.



BACTERIAL RESISTANCE

The sale of prescription antibiotics and their consumption must be supervised by the healthcare provider who prescribed it. This is because the overuse of this medication may lead to bacteria to get used to antibiotics or resistance. This means bacteria develop mechanisms that enable them to survive in the presence of antibiotics.

What consequences do you think the bacterial resistance may have?

After a bacterium has become resistant, what do you think is the way to treat a disease?

Discuss with others!

And vaccines... how are they related to this?

Vaccines are a biological preparation whose goal is to produce immunity against disease by stimulating the production of antibodies.

They can be, for example, a suspension of dead or attenuated microorganisms; products or subunits of microorganisms and even DNA. In general, as the response to vaccines is to generate antibodies, they usually stimulate the AIS.

A LITTLE BIT OF HISTORY

In history, there were several events that preceded the beginning of the era of vaccination. First, there were procedures where scabs caused by smallpox were inoculated in healthy individuals. Even though this procedure was performed from time immemorial, it was only in 1786 when the English physician and scientist, Edward Jenner, carried out the first medical experiment related to vaccination.

Jenner's experiment

Dr. Edward Jenner was a scientist, physician in the countryside and a poet. He observed that milkmaids that were in constant contact with cows and that occasionally got infected by a disease called vaccinia (kind of bovine smallpox), did not get smallpox that affects humans.

With this idea in mind, Jenner took secretion samples of the blisters from a milkmaid who has caught vaccinia (smallpox that affects bovines) and he inoculated the wound of a child with it. After six weeks, he inoculated the same child with pus from a person sick with smallpox and the child showed no sign of infection.

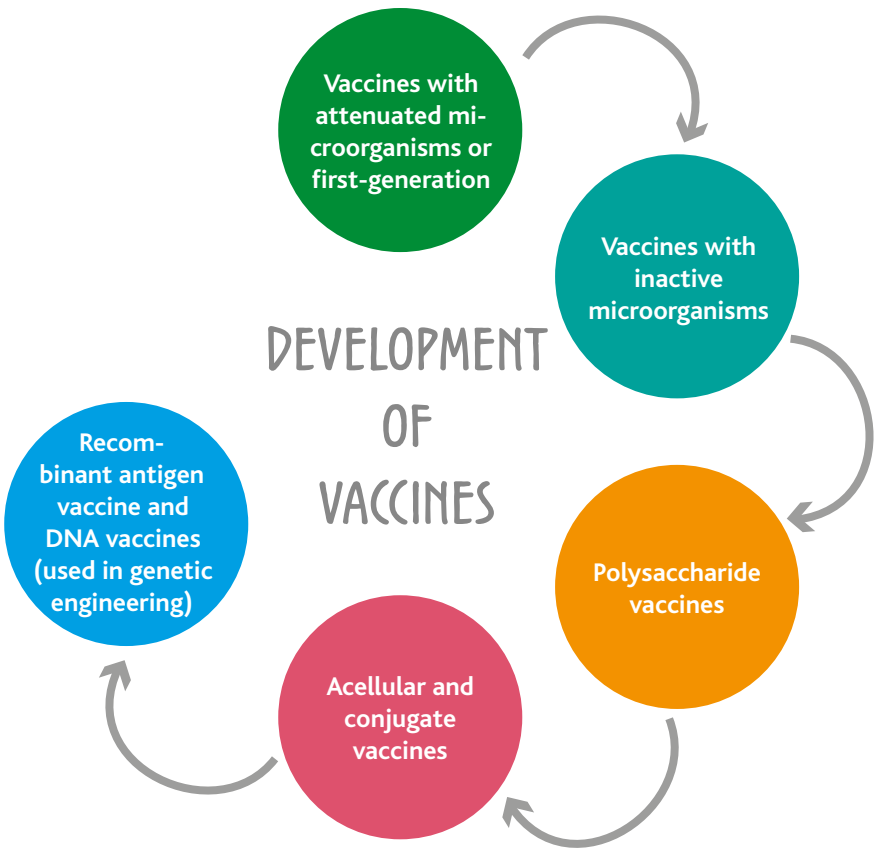
Later, it has been discovered that this cowpox is a milder variant of deadly smallpox that affects humans.

As a result of this, the term vaccination appeared.

Another important event related to vaccination occurred thanks to **Louis Pasteur**, a French chemist, and bacteriologist. He is considered the father of modern microbiology because of his countless contributions in this field. Pasteur created two vaccines for diseases caused by bacteria: the vaccine for chicken cholera in 1880 and the vaccine for anthrax in 1881.

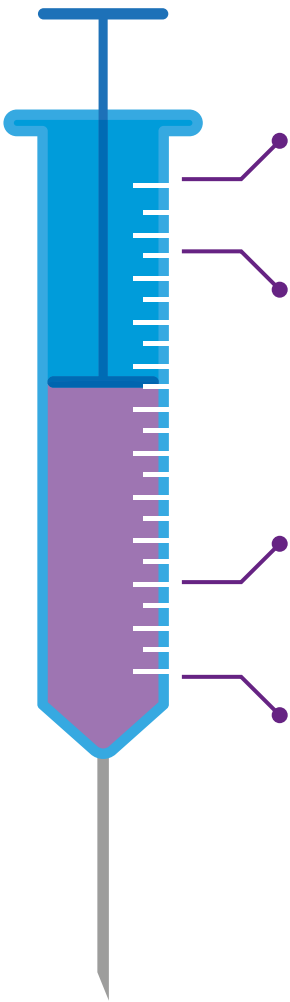
Later, in 1885, Pasteur creates the vaccine for rabies, also known as the rabies vaccine.

In this way, the experiments made by Jenner and Pasteur enabled the introduction of active vaccines. These vaccines were first developed with an attenuated vaccine method (known as first-generation vaccines); then, with the development of inactive microorganism vaccines; to continue with the polysaccharide vaccines and the genetic recombination mechanisms.



Types of vaccines

In the early development of vaccines, there were some predominated mechanisms of production. The technological advance has enabled the introduction of much more innovative and safer techniques.



ACELLULAR

A combination of purified subcellular components of the pathogen. They usually have proteins and it may have toxoids.

RECOMBINANT ANTIGEN

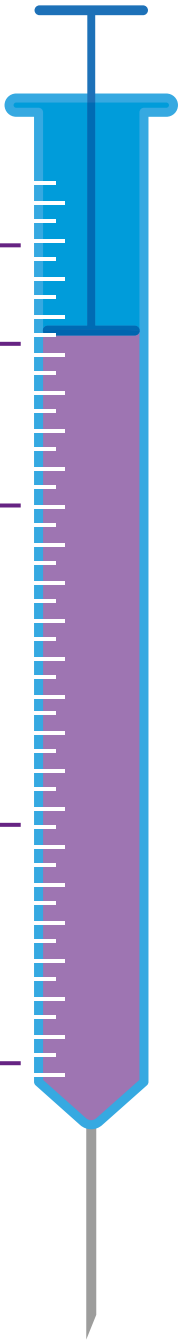
Using genetic engineering technology (recombinant DNA), a gene that encodes an antigen is inserted (a protein of a pathogenic microorganism) in a bacteria or yeast. The goal is to produce large amounts of the antigen protein for its subsequent purification and use.

CONJUGATE

In this type of vaccines, cell wall polysaccharides or pathogenic bacteria capsule are combined with proteins of the same pathogen. In this way, the vaccine develops an immune response against several antigens of the bacteria.

INACTIVE

Pathogenic microorganisms are treated with chemical products or heat. This type of vaccines activates the immune system, but it is not able to reproduce in the host. In this way, the immunity is milder and shorter, that is why more doses are needed.
In fact, because of the milder immune response of these vaccines, substances called adjuvants are used to increase the immune response of the organism.



POLYSACCHARIDES

External wall lipopolysaccharides or pathogen bacteria capsules are used to generate an immune response or recognition of the external part of the microorganism.

TOXOIDS

They are inactivated toxic components of pathogenic microorganisms.

DNA VACCINE

This type of vaccine contains the DNA of a pathogen that encodes antigenic proteins. One advantage of DNA vaccines is that they can be easily produced and stored, while the main problem is that it is not possible to know for certain if this DNA can integrate into a chromosome in the cells or cause mutations in the genome of the patient that receives the vaccine.

RECOMBINANT VECTOR

In this type of vaccines, it is administered an attenuated or nonpathogenic microorganism carrying DNA that encodes an antigen of the pathogenic microorganism. The most often microorganisms used in this type of vaccines are vaccinia virus, some nonpathogenic lactic acid bacteria and attenuated variants of M. tuberculosis and Salmonella Typhi. The main problem with this type of vaccines is the insufficient immune response.

LIVE ATTENUATED

These vaccines contain microorganisms cultivated under controlled conditions where they lost or attenuated their pathogenic properties. They develop longer immune response and are commonly used in adults.

Some important vaccines

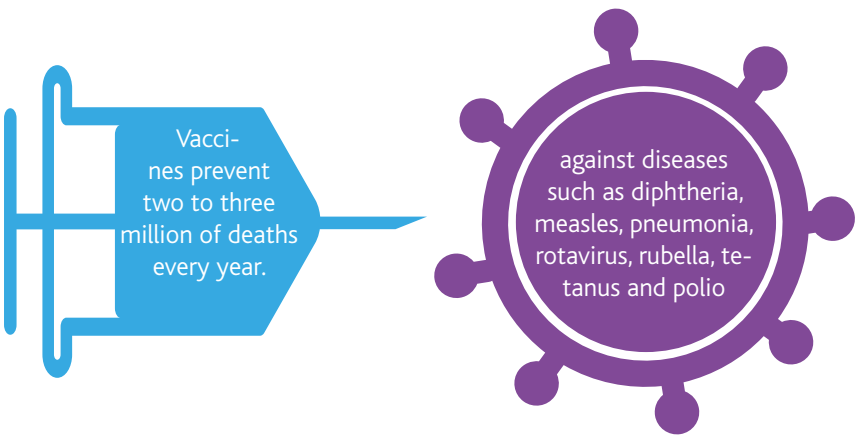
Year	Name	Type of vaccine
1895	Rabies vaccine	Live attenuated
1896	Cholera vaccine (*Vibrio cholerae*)	Inactive by heat
1896	*Salmonella enterica Typhi and S. enterica Para-typhi*	Live attenuated
1911	Rabies vaccine	Inactive
1926	Pertussis vaccine	Inactive with whole cells
1937	Yellow fever vaccine	Live attenuated
1937	Salk Anti-flu	Inactive
1950	Sabin Polio vaccine	Attenuated
1954	Salk Polio vaccine	Inactive
1960	Measles vaccine	Attenuated
1968 y 1972	Meningococcal A and C vaccine	Polysaccharides
1972	*Haemophilus influenzae* Type B (Hib) vaccine	Polysaccharides
1975	Typhoid fever vaccine	Polysaccharides
1978	Pneumococcal vaccine	Polysaccharides
1984	Chicken pox vaccine	Attenuated
1989	*Haemophilus influenzae* Type B (Hib) vaccine	Conjugate
1999	Meningococcal vaccine	Conjugate
2000	Heptavalent pneumococcal vaccine	Recombinant antigen

Age	Vaccine	Protects against
New born	BCG	M. tuberculosis Invasive disease
2 months	Pentavalent	Hepatitis B (virus) Diphtheria, Tetanus, Pertussis *H. influenzae* type b (Hib) invasive disease
	Pneumococcal conjugate	*S. pneumoniae* invasive disease
4 months	Pentavalent	Hepatitis B (virus) Diphtheria, Tetanus, Pertussis *H. influenzae* type b (Hib) invasive disease
	Pneumococcal conjugate	*S. pneumoniae* invasive disease
6 months	Pentavalent	Hepatitis B (virus) Diphtheria, Tetanus, Pertussis *H. influenzae* type b (Hib) invasive disease
	Pneumococcal conjugate (only for preterms)	*S. pneumoniae* invasive disease
12 months	Meningococcal conjugate	*N. meningitidis* invasive disease
	Pneumococcal conjugate	*S. pneumoniae* invasive disease
18 months	Pentavalent	Hepatitis B (virus) Diphtheria, Tetanus, Pertussis *H. influenzae* type b (Hib) invasive disease
	Meningococcal A and C	Polysaccharide
First grade	dTp (acellular)	Difteria, Tétanos, Tos convulsiva
8th grade	dTp (acellular)	Difteria, Tétanos, Tos convulsiva
Adults aged 65 years old and older	Pneumococcal	Enfermedades invasoras por *S. pneumoniae*

Chilean Health Department Immunization Schedule to prevent bacterial diseases

What is the importance of vaccines?

A region or country performs a massive vaccination campaign in order to protect people against infectious diseases. In fact, the World Health Organization (W. H. O.) estimated that vaccines prevent two to three million of deaths every year, by providing protection against diseases such as diphtheria, measles, pneumonia, rotavirus, rubella, tetanus and polio. However, for some years now, people have started forgetting these benefits.



The **anti-vaccination groups or movement** are present in developed and developing countries and have similar arguments. They oppose vaccines arguing that they may cause side effects, disorders such as autism or ischemic strokes with time.

The problem is that their arguments are based on wrong scientific sources. The one that has caused major impacts is a study published in The Lancet, in 1998. This study suggested a connection between the development of autism and the triple vaccine (measles, rubella, and mumps). The journal retracted the paper when it was proved that the author had falsified the results.

Even though infectious diseases are uncommon in some countries (mainly developed countries), the microorganisms that cause these diseases continue to spread. And in a globalized world, they can cross geographical boundaries and infect any unprotected person, so the anti-vaccination option, it doesn't only affect the person who decides, but also affects his entire community.

What if I don't get vaccinated?

A person who doesn't get vaccinated becomes a reservoir for the pathogenic microorganism and thereby helps spread it.

In 2013, in Wales (United Kingdom) there was a measles epidemic with more than a thousand children, between 10 and 15 years old, infected. This happened because part of the population decided to not get their children vaccinated.

Remember! The real or false information goes viral as fast as a microscopic infection, so get vaccinated against it and do some research to know the truthfulness of the sources. Assess information to know if it is true, check the references and don't be fooled by the sensationalism of magazines, blogs, opinion columns or socials networks that don't include the references of scientifically proven studies. Be careful with fake news.

PERSONAL HYGIENE

Prevention is the best way to avoid catching bacterial infectious diseases.

Some measures are:

Vaccination

The first step is to get vaccinated. The Chilean Health Department Vaccination Schedule is designed to prevent infection due to bacterial (such as diphtheria, tetanus, and pertussis) and viral (such as hepatitis) diseases. Having your vaccination record up to date allows preventing infection and the spread of these diseases.

Hygiene

Washing hands is an effective method to prevent different diseases. That is why, it is recommended to do it regularly and properly, which means; first wet your hands; then lather your hands by rubbing them together with the soap; and finally scrub your hands for at least 30 seconds.

You need to wash your hands several times a day. For example:

- every time you go to the bathroom
- after sneezing or blowing your nose
- after playing or being with animals
- before every meal

The oral cavity may also be a place where pathogenic bacteria proliferate, like those causing cavities. For this reason, brushing and flossing your teeth is essential to maintain a good oral health

Washing fruits and vegetables

Fruits and vegetables, especially those that we eat raw, we must properly soak, wash and rinse to eliminate dirt, insects and bacteria residues. When they are not properly clean, you may get a gastrointestinal disease.

Cross-contamination

In the kitchen, raw food must be separate from cooked food, and you must use different utensils to cook each type of food. In this way, we prevent "cross-contamination". In addition, it is essential to properly wash the kitchen utensils, especially those used to handle raw meat. Bacteria present in the meat die when the meat is cooked, but if raw vegetables are prepared with the same unwashed utensils, it is possible those bacteria cause an infection or food poisoning.

CHAPTER 4

DISEASES CAUSED BY BACTERIA

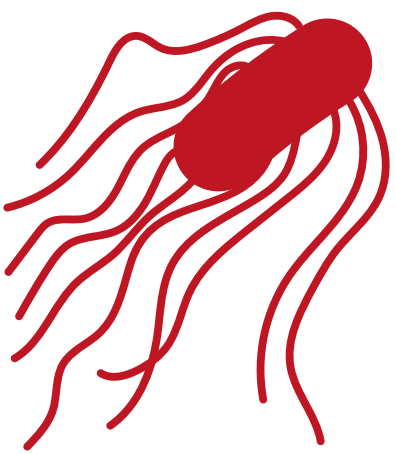
Before starting:
What diseases caused
by bacteria do we know?
Share with others!

Diseases caused by bacteria

GASTROINTESTINAL DISEASES

Las infecciones intestinales conocidas como gastroenteritis, son causadas por Intestinal infections, known as gastroenteritis, are caused by bacteria, viruses or parasites that harm the intestinal mucous causing diarrhea, vomiting, abdominal pain, and fever.

Depending on the intensity of the medical condition or the type of infection, the patient may become dehydrated. Children and the elderly are at risk for dehydration. Infectious diarrhea is considered one of the most serious health problems in undeveloped countries, being one of the main causes of childhood diseases and mortality. The main microorganisms causing foodborne infections are species of *Salmonella* and *Campylobacter, Escherichia coli* and *Listeria monocytogenes*.

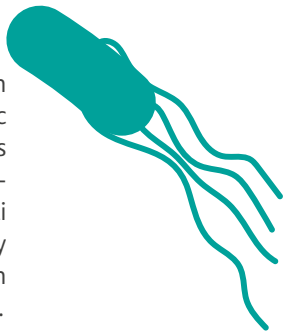


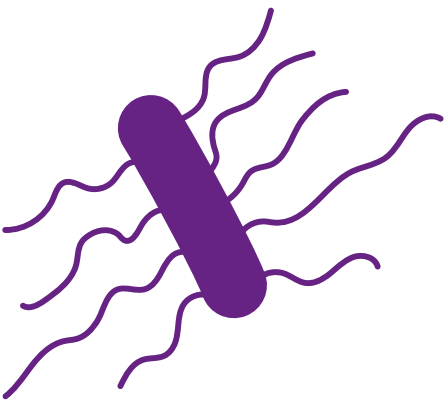
SALMONELLA

This bacterium causes a disease called, "salmonellosis" when the pathogen grows in the digestive tract. The symptoms include sudden headaches, fever, chills, vomiting, and diarrhea. Salmonellosis may be caused by eating food contaminated by the bacterium, such as vegetables, raw or not fully cooked meat, mayonnaise, raw eggs, etc.

ESCHERICHIA COLI

This bacterium is commonly found in the human and other animals' intestine. It can be pathogenic when the bacterium contaminates food and this food is eaten. The symptoms can be diarrhea, vomiting, and fever. One strain of E. coli, called E. coli O157:H7 or enterohemorrhagic (EHEC) is very dangerous and it causes bloody diarrhea. This strain can result in kidney failure by the effect of a toxin.



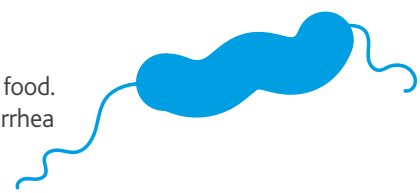


LISTERIA MONOCYTOGENES

These bacteria enter the body through the gastrointestinal tract. Here, the phagocytes internalize them, which enable bacteria to proliferate and spread, because it is an intracellular pathogen. When it goes into the bloodstream, it can cause sepsis or meningitis.

CAMPYLOBACTER

Humans can get it by eating contaminated food. It is the most common cause of bacterial diarrhea in children.



VIBRIO PARAHAEMOLYTICUS

This bacterium can be found in estuaries, seawater, and some organisms, such as filter-feeding bivalve mollusks (ribbed mussel, abalones, mussel, clams, oysters, etc.). This bacterium causes gastroenteritis with an incubation period of 12 to 48 hours. In general, the infection tends to run its course in two to three days.

GONORRHEA

Gonorrhea is one of the most common sexually transmitted infections and any sexually active person can get infected.

It is caused by the Gram-negative bacterium, *Neisseria gonorrhoeae*, which mainly infects the urogenital mucosa.

The bacterium adheres to mucous cells, enters them and proliferates. Then, it enters the subepithelial space where produces the infection. Structures in the bacterial surface, such as pili or fimbriae, and adhesins proteins, are important for the initial adhesion.

Effect in women

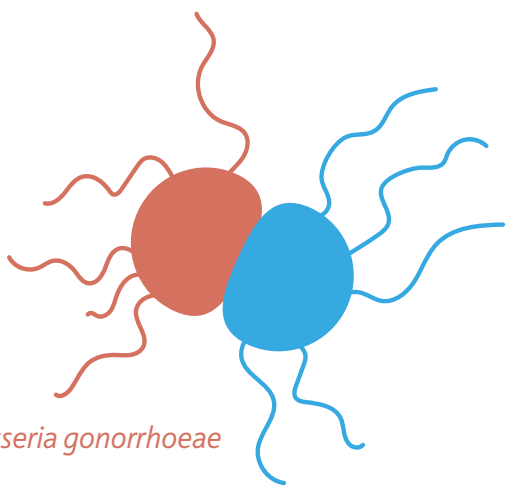
In women, the *N. gonorrhoeae* can be found in the reproductive tract (including Fallopian tubes, uterus, and cervix), and the infection may be asymptomatic.

An untreated gonococcal infection can cause acute pelvic inflammatory disease due to the colonization of the bacteria. It causes an inflammation of the uterus, Fallopian tubes, and abdominal cavity.

The lesions and the formation of scar tissue caused by untreated gonococcal infections may lead to infertility.

Effect in men

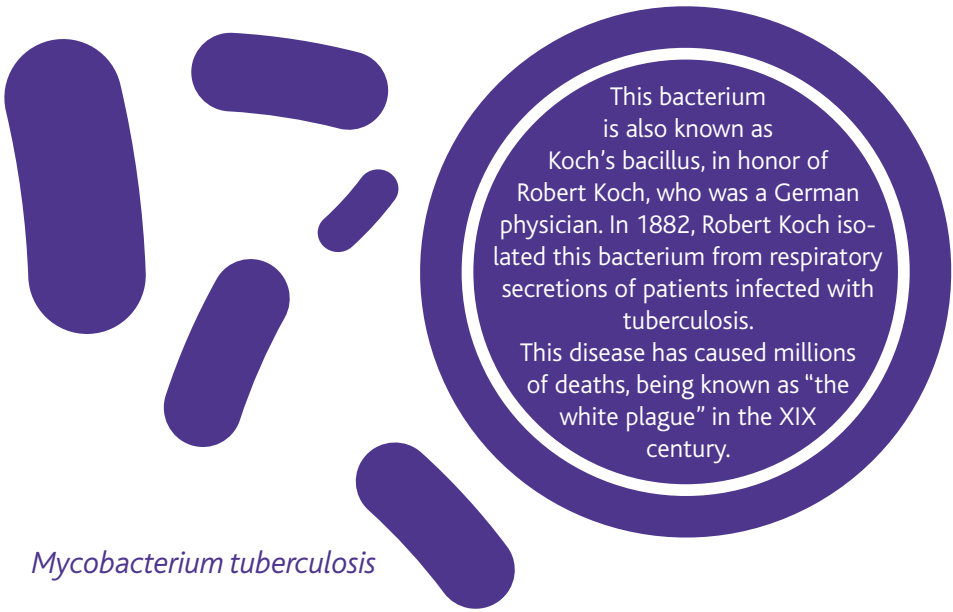
In men, the bacterium might cause scar tissue in the urethra, making it more difficult for urine to flow.



Neisseria gonorrhoeae

TUBERCULOSIS

Tuberculosis is a contagious bacterial infection caused by *Mycobacterium tuberculosis*, which mainly affects the lungs, but it might spread to any organ.



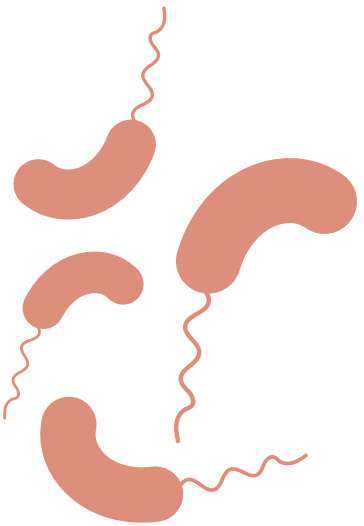
Mycobacterium tuberculosis

M. tuberculosis can be transmitted by breathing in air droplets or aerosols from a cough or sneeze of an infected person. The bacteria stay and proliferate in the lungs.

A type of delayed reaction produces activated macrophage nodules in the lungs, called tubercles. However, bacteria tend to survive and even proliferate in the tubercles. Then, an acute infection is produced, which may lead to the destruction of the pulmonary tissue; the spread of the bacteria to other parts of the body; or death.

CHOLERA

Cholera is an acute diarrheal infection caused by the bacterium *Vibrio cholerae*. The most severe cases of cholera may lead to dehydration.



Molecular causes of Cholera

The symptoms are caused by a toxin that activates the membrane enzyme called adenylate cyclase. This enzyme converts adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP), which is a molecule involved in different regulatory processes in the cells, such as hormones activity, synaptic transmission in the nervous system and inflammatory and immune responses in the tissues.

High cAMP levels increase the secretion of chloride ion and bicarbonate into the lumen. The ionic balance is disrupted causing water secretion into the intestinal lumen.

In the acute phase of cholera, the loss of water from the small intestine is greater than the reabsorption in the large intestine, resulting in a major loss of fluids. Approximately, five percent of people infected with V. cholerae may present a severe cholera disease, which is characterized by profuse watery diarrhea, vomiting, and leg cramps. In these patients, the rapid loss of body fluids may lead to dehydration and prostration. The lack of proper and timely treatment can lead to death within hours.

A person can get cholera by eating food contaminated with the cholera bacterium. In an epidemic, the source of the contamination is usually the feces of an infected person.

URINARY TRACT INFECTIONS

The microbial colonization and spread in the urogenital epithelium cause **urinary tract infections (UTI)**. It is usually a bacterial infection and affects the bladder causing cystitis.

Most of the bacteria that cause urinary tract infections are Enterobacter species, particularly Escherichia coli and *Proteus vulgaris*. However, nosocomial diseases may be caused by other Gram-negative or Gram-positive bacteria.

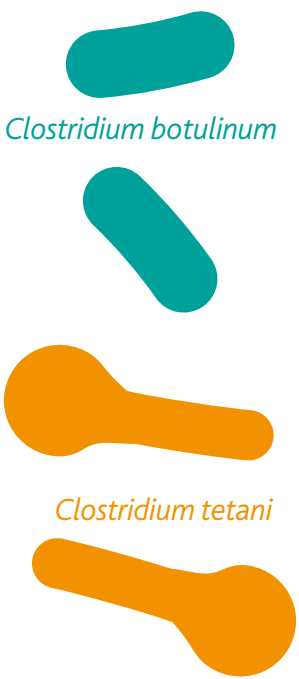
A UTI can be easily treated with the proper antibiotic. However, in severe cases, the infection may spread to the kidneys and cause pyelonephritis.

OTHER IMPORTANT DISEASES

BOTULISM

Clostridium botulinum and *Clostridium tetani* bacteria are naturally found in soil and sometimes cause diseases in animals. They can grow and proliferate when food is improperly canned.

The disease caused by this microorganism is produced by a toxin that binds to the presynaptic neuron membrane and inhibits the release of acetylcholine. The transmission of the nerve impulse to the muscle is propagated by acetylcholine. For this reason, the toxin causes flaccid paralysis, meaning that the muscles are unable to contract. The mortality rate of this disease is close to one hundred percent, depending on the amount of the toxin ingested, and it can be fatal mostly because of respiratory failure as a result of muscular paralysis.



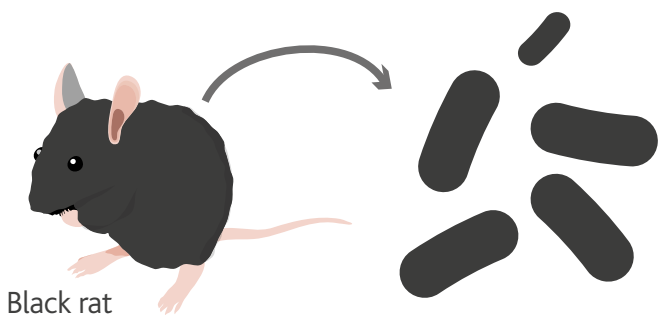
BLACK DEATH

In this disease, the bacterium *Yersinia pestis* causes swollen lymph nodes in the groin, armpit, and neck. Other symptoms are suppuration and fever, producing chills and delirious in many people.

In the 14th century, the most common form of the disease was swollen lymphatic nodes. At that time, this organ was called bubo, and therefore the disease was called bubonic plague.

Y. pestis colonized rodents, particularly black rats, and it was transmitted through fleas (*Chenopsylla cheopis*) that lived on them. The fleas, by biting humans, inoculated the bacteria into humans. The frequent contact between them facilitated the infection and spread of the disease.

It is estimated that between 1347 and 1353, the Black Death reduced the European's population from 80 million to only 30.



Black rat

Yersinia pestis

There were also other forms of the disease, such as the septicemic plague. In this case, the bacterium multiplies in the bloodstream. The signs of this disease were dark skin patches, which were the origin of the name "Black Death," or "Black Plague." The pneumonic plague affected the respiratory system and the symptoms included a productive cough that could cause airborne transmission.

LEPROSY

This disease is characterized by skin lesions, especially in the face and extremities, caused by the *Mycobacterium leprae* bacteria. This pathogen, which grows inside the macrophages, causes an intracellular infection that may lead to the bacteria proliferation in the skin, and, as a result, a large skin lesion. In severe cases, the lesions cause deformities that may lead to the loss of the motor function.

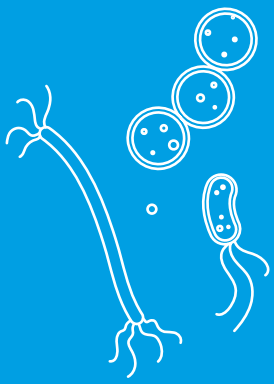
The transmission of this disease involves direct contact and inhalation of the microorganism. Incubation times range from weeks to decades and years.

Mycobacterium leprae

TETANUS

Tetanus is a disease caused by a microorganism called *Clostridium tetani*. Its natural environment is the soil and occasionally causes illness in animals. The bacterium can infect a deep wound and secrete a toxin that reaches the neurons. Here, it causes severe neurological symptoms that can be fatal since it generates an uncontrollable contraction of the muscles. If the respiratory muscles are affected, it may lead to death by suffocation.

Clostridium tetani



CHAPTER 5

CURRENT DISCUSSIONS

Current discussions

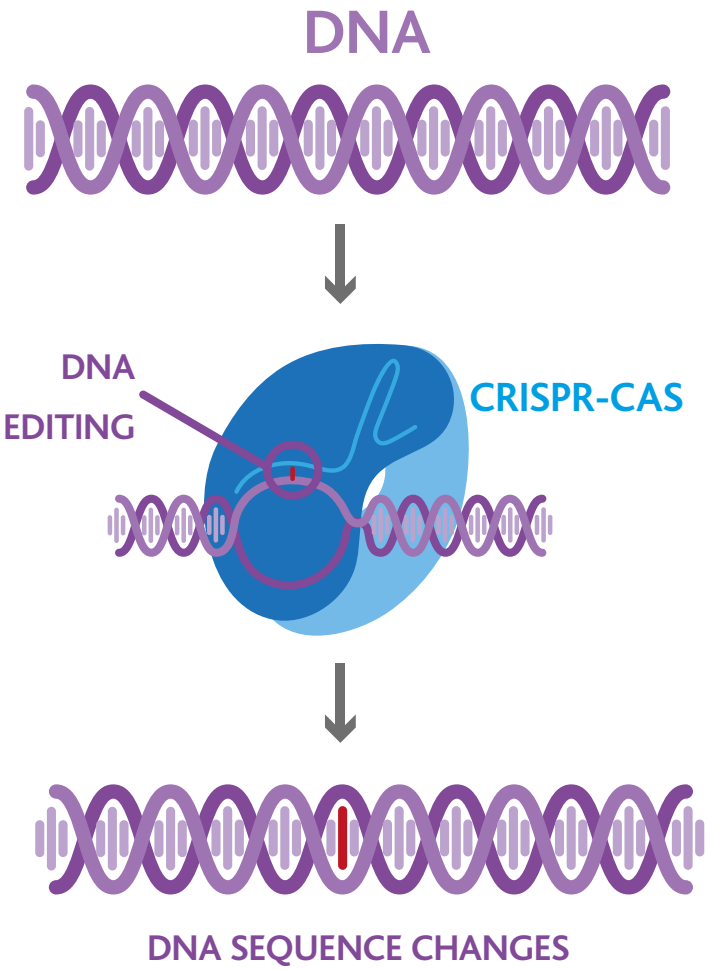
GENOME EDITING

For decades, people have been yearning for being able to permanently alter the DNA, by inserting or deleting genes or specific pieces of DNA. The reason is that this process can be applied to agronomy, veterinary and human medicine.

Some tools that are currently used to modify DNA are Zinc finger nucleases (ZFNs), transcription-activator like effector nucleases (TALEN), and the revolutionary technique of nucleases and the clustered regularly interspaced short palindromic repeats (CRISPR/Cas) system.

The first two, are based on proteins conformed by a DNA catalytic domain and a recognition domain of the targeted gene. It is not possible to make multiple changes simultaneously with the first two techniques. However, with the CRISPR/Cas technique, it is possible to modify a DNA sequence easily, quickly and accurately in different domains of the genome of a living organism. This genome editing technique is inspired by the rudimentary immune system with which some bacteria keep virus genome fragments in their genome. This process allows the bacteria to identify the virus and produce enzymes that cut and deactivate the RNA virus.

The CRISPR-Cas technique has already been used to cause and correct mutations in different types of cells, including bacteria and human eukaryotic cells. However, its main inconvenient is the involuntary generation of genetic material errors. This situation decreases the efficacy of the technique, preventing it to be considered as a future gene replacement therapy.



A new CRISPR version developed by Gaudelli et al. reduced the involuntary genomic error rate from 10 to 0.1 percent. The researchers have proved their new technology by correcting genetic errors on blood pathologies (leukemia) which were originated by DNA mutations in the eukaryotic cells.

Chilean scientists in vaccine development

DR. PABLO VALENZUELA

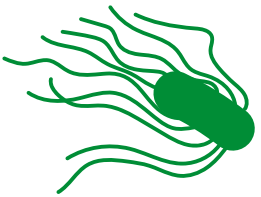
Dr. Pablo Valenzuela discoveries changed the face of modern medicine. He is a restless scientist (Biochemist of Universidad de Chile. Chemistry Ph.D. of the Northwestern University, and postdoctoral researcher at the University of California), and an entrepreneur by nature (he founded the biotechnology companies Chiron Corporation and BiosChile). Dr. Valenzuela developed the vaccine against Hepatitis B, sequenced the genome of the immunodeficiency virus, and under his direction, the Hepatitis C virus was discovered. He currently works at the Foundation Science and Life, searching for new drugs to treat cancer.

DR. ALEXIS KALERGIS

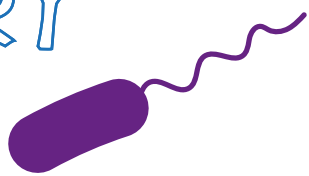
In an interview with CONICYT, Dr. Kalergis said that "this pioneering initiative is transformed into a phase I clinical study, the first for a vaccine developed by Chilean science and in compliance with all national and international regulations."

Dr. Alexis Kalergis, professor of the Pontificia Universidad Católica de Chile, developed in Chile a vaccine against the respiratory syncytial virus (RSV). This virus is the main cause of respiratory infectious diseases in children in Chile and the world. The vaccine has passed the pre-clinical and manufacture studies, and there is an agreement with the Health Department to ensure access to the vaccine for the entire population through the National Program of Immunization.

Also, in collaboration with Dr. Susan Bueno, Dr. Kalergis is developing a vaccine against the human metapneumovirus (HMPV), which causes respiratory infectious diseases, especially among children and older adults.



GLOSSARY



Antibody: molecules produced by B lymphocytes. The antibodies, (Ab) have a common basic structure. However, the antigen-binding site is specific to each of them.

Antigen: any molecule able to induce the production of specific antibodies and the activation of B lymphocytes.

B lymphocytes: are a type of lymphocytes responsible for creating or producing antibodies for a specific antigen. They can also work as antigen-presenting cells, and, eventually, they can transform into memory B Cells when activated by the interaction with an antigen.

Bacteriocins: proteins with antibacterial activity.

Complement system: This system is part of the innate immune system and is a nonspecific defense against pathogenic microorganisms. It consists of proteins called the complement. In the case of inflammatory reactions, the complements travel to the infected tissue and here, they work as a sign to attract immune system cells. They adhere to harmful bacteria to mark them as a target or they form pores in the membranes of the pathogens and cause lysis.

Eukaryote: cells that have a nucleus that contains the genetic material (DNA). They are complex and evolved cells. Inside, there are membranous organelles. This cell type can be animal or plant.

Gram's method or Gram stain: It is a method created by Christian Gram in 1884 to distinguish and differentiate bacteria under the microscope. The first step is the application of a crystal violet stain. Then, there is the decolorization with ether and/or acetone. Gram-positive bacteria retain the first stain, causing it to look violet or dark blue under a microscope. Gram-negative bacteria become decolorized. Finally, a secondary stain (safranin) is added and Gram-negative bacteria will appear pink.

Immunological memory: it is the ability of rapid immune response against an agent that already had contact with the immune system.

Integrases: recombination enzymes that recognize short specific homologous sequences.

Interneuron: is a central nervous system neuron that interacts with other neurons exclusively, and never with sensory receptors or muscular fibers.

Lipopolysaccharides: They are the main component of the outer membrane of Gram-negative bacteria. They are the main responsible for the endotoxin shock induced by Gram-negative bacteria.

Lymphocytes: or also called white cells are the blood cells responsible for the specific defense of the immune system. They have receptors in their membranes that allow them to recognize a large variety of pathogens.

Macrophages: antigen-presenting cells. After ingesting foreign particles or antigens, the macrophages present them to the lymphocytes to begin maturation and specific immune response.

Motor neurons: neurons of the central nervous system that project their axon towards a muscle or gland.

Mucosae: is a layer of cells that secretes substances containing protective elements against pathogenic bacteria. They are present in the digestive, respiratory and urogenital tract.

Nosocomial infections: infections contracted by patients as a result of being hospitalized. They are also called hospital-acquired infection.

Palindromic sequence: is a DNA or RNA sequence arranged in a way that it reads equally from 5 'to 3' and from 3 'to 5'.

Peptidoglycan: it is the main component of the bacterial cell wall. It can be defined as a structure consisting of sugars and amino acids that surrounds the cell membrane, giving structural strength.

Phylum: is the third element of classification of living beings in a taxonomic hierarchy. The first one is "Domain", then "Kingdom", and finally "Phylum" or "Division". Phylum groups together all living things with the same organization system.

Prokaryote: very simple cells that lack membrane-bound organelles or nucleus. This is why the genetic material (DNA) is located in the cytoplasm. Bacteria belong to this type of microorganisms.

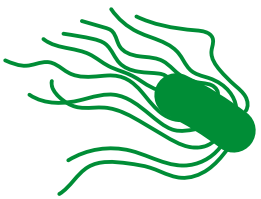
spp.: in general, is an abbreviation to refer to all the species of a genus.

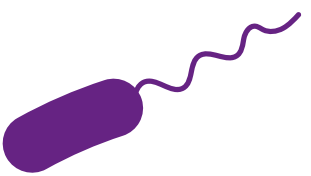
T lymphocytes: also called T cell. They are an important type of lymphocytes that maintain the immune system and are essential to fight against pathogenic microorganisms. There are two main types of T lymphocytes, Cytotoxic T cell (Tc) and the T helper cells (Th). The Tc look for antigen infected cells in the body. When a Tc recognizes an antigen bound to a cell, it adheres to the surface of the infected cell and produces toxic molecules, killing the cell.

The Th secretes cytokines when activated by an antigen. Then, they activate B lymphocytes to begin their immune response

Transposons: Are mobile DNA fragments that can move from one chromosome to another.

Virulence factors: It refers to pathogen's intrinsic properties that enable adhesion, invasion, and infection of the host. Adhesins are bacterial adhesion factors that enable pathogen bacteria to colonize the host's mucus. Some components of the surface or enzymes, facilitate host cell invasion. Toxins change the physiology of the cell or the host, resulting in the appearance of the symptoms and the development of the disease.





SCIENCE EXPERIMENTS

Let's get started!

Learn by knowing and experimenting in the microworld of bacteria. Helped by an adult, perform these activities.

Activity N°1

"Growing microorganisms in Petri dishes"

Materials:

- 1 envelope of plain unflavored gelatin
- ½ liter of hot boiled water (2 ½ cups or 500 mL)
- 1 beef bouillon cube
- A 3 cups or 600 mL size bowl
- 2 cups
- Plastic or glass Petri dishes (You can also use short plastic boxes with lids)
- 10 cm ruler

Dissolve the beef bouillon cube in 1/4 cup of water (50 mL). At the same time, dissolve the envelope of plain unflavored gelatin in 3/4 cup of water (150 mL). Stir both mixtures, dissolve and add 1 cup of water (200 mL).

Pour the resulting medium into the Petri dishes (or short plastic boxes with lids) until reaching 0.5 cm height (use the ruler to measure). Cover the Petri dishes and allow them to harden stored in a refrigerator. The content of the Petri dishes will be used in activities 2, 3, 4 and 5.

With the final mixture of this activity, you should be able to prepare around 20 Petri dishes of 90 mm in diameter.

Activity N°2

"Growing environmental de microorganisms"

Materials:

- Cotton swabs
- 2 Petri dishes
- Permanent marker

Grab a cotton swab and swipe it over the surface of any object around you, such as a table, chair, toilet, cell phone, etc. This process is called sample collection or sampling.

Then, apply the sample to a petri dish from activity N°1, rubbing the swab in a zigzag pattern. This process is called inoculate or plate.

Then, place the lid on the Petri dish and label it over the external side of the dish, writing down the place where the sample was collected, the name of the person collecting the sample and date. Do not label the lid. This process is called labeling. Repeat the steps of sampling, plate, and label for each place you want to analyze.

The incubation process consists in placing the inoculated plates in a place where bacteria can grow. Leave the plates upside down at room temperature for 48 hours and in a place with no direct light. Then, analyze the colonies present in the agar and compare color, texture, form, etc.

After two days, you can store the plates in a refrigerator (4 a 6°C) for a couple of days.

Activity N°3

"Growing microorganisms that live in our body"

Materials:

- Cotton swabs
- 2 Petri dishes
- Permanent marker

Using a swab, take a sample from inside the nose of a classmate. Plate the sample in a Petri dish following the instruction in activity N°2.

Do not forget to label the Petri dish! At the same time, use another swab to take a sample from inside the mouth (tongue or cheek) of another classmate and repeat the procedure.

Incubate the labeled plates in a warm place, with the agar facing upwards and with no direct light during 48 hours. Then, observe the microorganisms growing in each of the plates. Analyze the colonies comparing color, texture, form, etc. After two days, you can store the plates in a refrigerator (4 a 6°C) for a couple of days.

Activity N°4

"Optimal culture media for microorganisms"

Materials:

- Three 17 oz clean plastic bottles
- 3 cups of warm boiled water
- 1 beef bouillon cube
- Bowl to dissolve the bouillon cube
- 1 spoon (15 mL)
- Vinegar
- Salt
- Permanent marker

Dissolve the beef bouillon cube in 3 cups of water. Distribute the content in the three bottles (1 cup per bottle). In the first bottle, add 5 tablespoons of salt. In the second one, add 5 spoons of vinegar. And in the third one, do not add anything. The third one will be the control bottle. Label each bottle to easily distinguish them and place them in a warm place. Incubate for five days. After the incubation period, analyze the bottles and compare their turbidity or lack of transparency. Higher turbidity levels indicate greater growth of microorganisms.

Activity N°5
"Microorganisms respiration"

Materials:

- One 17 oz bottle, ideally a plastic one
- 1 balloon
- 1 envelope of bread yeast (Saccharomyces cerevisiae)
- 2 tablespoons of sugar
- Warm water
- 1 pitcher (or similar)
- 1 spoon (15 mL)
- Tape

In the pitcher, pour 1 ½ cup of warm water and add the yeast (1 envelope). Mix everything together. Then, add 2 tablespoons of sugar. Stir until having a homogeneous mixture and pour the content into the bottle.

At the same time, blow up the balloon to make it more elastic. Then, deflate it and put it in the mouth of the bottle. Fix it with tape. Finally, place the bottle with the balloon on a flat surface.

Incubate during some hours and observe and register the processes that happen with the balloon and the yeast dissolved inside the bottle.

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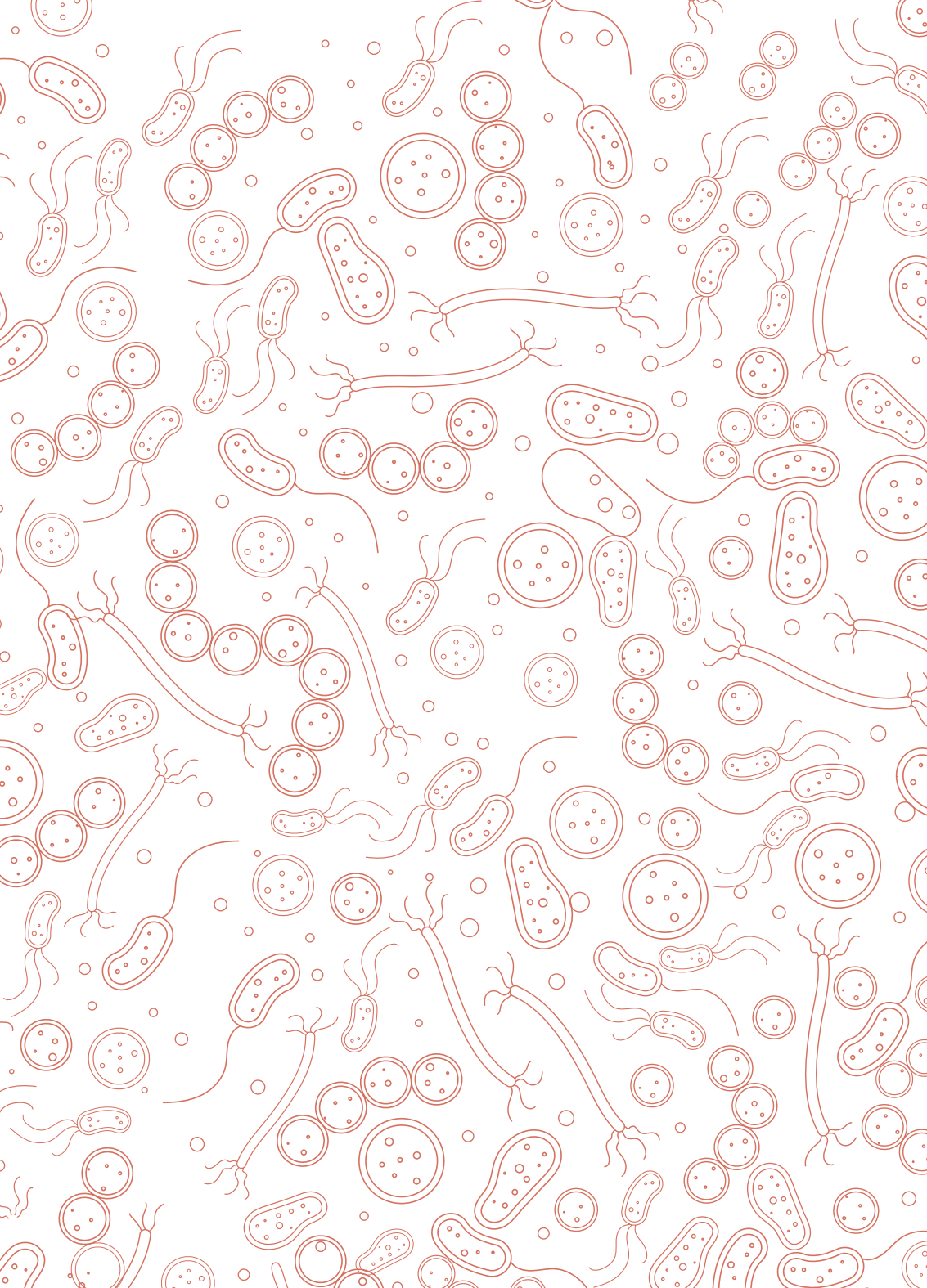

Bacteria, why do they make me sick? is a book that aims to familiarize us with the tiny and invisible world of the microorganisms we live and constantly interact without even noticing it.

This document provides exploratory and scientific outreach material for curious readers of all ages. It focuses on students and teachers, for whom, ***Bacteria, why do they make me sick?***, may be a useful, teaching, and visually appealing educational tool.

DUC IN ALTUM

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