

The Tale of a Bacteria Battle

A study on Staphylococcus aureus, its prevalence, possible clinical symptoms and the tools we have available to fight it

KFL082

3938 words

Contents

1	Introduction	3
2	Theoretical context	5
2.1	Bacteria and bacterial infections	5
2.2	The enemy: <i>Staphylococcus aureus</i>	6
2.3	The enemy's attacks	8
2.4	Our weapons	8
3	Experimental design	13
3.1	Variables studied	14
3.2	Bill of materials	14
3.3	Biosecurity and risk mitigation	15
4	Physical experimentation	17
4.1	Description	17
4.2	Protocol followed	17
4.3	Results and analysis	18
5	Conclusions	23
5.1	Bibliographic conclusions	23
5.2	Experimental conclusions	23
5.3	Strengths and weaknesses	24
5.4	Possible improvements	25
I	Appendix	III
Annex 1 – Informed consent given to subjects before sampling		V
Annex 2 – GDPR notice given to subjects before sampling		XI

Annex 3 – Bibliography consulted (but not referenced)**XV**

1

Introduction

Good writing starts strong. Not with a cliché, not with a banality, but with a contentful observation that provokes curiosity.

Stephen King

A few years ago, in summer of 2021, I was accepted into a program at the Barcelona Autonomous University, aimed to divulge microbiology and biotechnology to a group of 50 biology-loving students. That's where I learned bacteria in detail, as well as how a microbiology/biotechnology lab functions. I fell in love with the discipline at first sight. I wondered how research in this field works. I took one of the experiments we did, and decided to expand it for my EE. This extended essay has as primary objective answering these two questions:

"What is the prevalence of Staphylococcus aureus in our school?"

"Is the prevalence of Staphylococcus aureus affected by gender or age?"

And it has as secondary ones studying bibliographically *Staphylococcus aureus*, improving my lab etiquette, protocol-making

This study requires taking samples from human subjects. This is a one-off sampling process: the subjects are required only once. The results are then communicated to the subjects via e-mail or by being delivered a physical piece of paper. They are informed previously on the process they are going to go through, as well as the purpose of the experiment. Each subject must read and agree to two documents: an informed consent which explains everything about the experiment¹ and a GDPR notice which

¹See annex 1

documents the use of their data as well as an expected timeline for data anonymization and destruction². All participants were screened to be over the age of 16, in order to ease the process and require no previous authorisation by parental figures on the data collection. The experimentation followed has no effect on the subjects[1].

Since bacteria were used, some aspects of the experiment must be clarified and discussed. Previously to starting the experiment, I read profusely the WHO's Laboratory Biosafety Manual and Associated Monographs (4Th Edition)[2] in order to find ways to mitigate any possible risk. During the experimental phases, there were no accidents or incidents. All plates were accounted for and controlled closely. No person other than me was allowed to come in contact with a plate that had been cultivated or with any used but not disinfected auxiliary material. The cultivated plates were considered Biosecurity Level 2. All possibly infected material was disposed of taking into account the risks that the bacteria in question posed, using fresh bleach.

Before starting the experimentation, and following the IBO EE guidelines, I talked with my coordinator in order to solidify the fact that there was no alternative to sampling from humans, and consider the risks that this experiment implies because of bacteria.

²See annex 2

2 Theoretical context

Each source that I read, I would look through the bibliography and the footnotes, and use that as a map for the next thing I would read.

Alexander Chee

§2.1 Bacteria and bacterial infections

Bacteria are prokaryotic organisms, generally single-celled, which are part of the Monera kingdom. Their sizes range from between $30\mu\text{m}$ and $100\mu\text{m}$ and are ubiquitous¹ organisms. This form of life is believed to be the first one to have ever appeared on Earth, as well as the one responsible for the oxygen-rich atmosphere the Earth currently has. Some species are hard to culture in a laboratory environment, but generally, those that can be cultured in a controlled environment are grown in agar plates[3].

Pathogenic bacteria are bacteria that have the ability to cause disease². These are not the most common type of bacteria, as the majority of them are either harmless or beneficial to the human body through symbiosis, such as the bacteria that help with digestion in the stomach[3].

¹Ubiquitous: found everywhere

²A disease is a particular abnormal condition that negatively affects the structure or function of all or part of an organism, and that is not immediately due to any external injury[4].

§2.2 The enemy: *Staphylococcus aureus*

Staphylococcus aureus (also known as Staph) is a GRAM-positive bacteria, the most studied and one of the most prevalent of its genus. Staph bacteria are usually harmless. However, they can, in some cases, cause serious infections that, in some cases, can lead to sepsis or death. Some of its distinctive characteristics include having a very thick glycopeptide wall, which allows it to withstand extreme temperatures and osmotic pressures, therefore rendering most classic methods of food conservation (such as cooking, smoking, freezing, or salting) completely useless against said bacteria; a protein A capsid, which binds to many eukaryote organisms; as well as thermoresistant enterotoxins. It's an extremely resistant (and thus ubiquitous) bacteria. It can be found in human skin, especially below the nails, and mucous surfaces (such as the mouth or the nose), as well as in certain foods such as ham (even after it's been cooked or curried), eggs, poultry and both raw and cooked dough.

Staphylococcus aureus has three main parts to its virulence: its cell wall, its membrane-bound factors and its secreted factors. Staph's **cell wall** is made up of three parts, going from inside to the outside of the cell: a plasma membrane, a peptidoglycan layer and a slime (sometimes also called capsule) layer[5]. The plasma membrane consists of a lipid bilayer that is semipermeable, which regulates the transport of materials entering and exiting the cell. Integrated inside them are a type of integral protein called penicillin-binding protein (PBP), amongst other proteins such as protein channels. We will only talk about PBPs because they are the Achilles's Heel of bacteria, as long as one has the proper tools to exploit it. Whilst the name implies PBPs are only sensible to penicillin, the name actually came to be this way because it's how they were discovered, and in fact could be resistant to it but sensible to other similar antibiotic agents. Variations in this protein may lead in some cases to antibiotic resistance, such as MRSA (*Methicillin-Resistant Staphylococcus aureus*), a variation of Staph that is the result of a variation in this protein called PBP2A[6]. The different variations of *Staphylococcus aureus* will be discussed in more detail in a following section.

Staphylococcus aureus, like all other members of the *Staphylococcus* family, have very thick peptidoglycan layers. This grants them protection from extreme temperatures and high osmotic pressures, which means these bacteria can colonise cooked food and food that has been salted. The most notable example is ham, either cooked, smoked or cured. Since little to no other bacteria can survive in those conditions, *Staphylococcus aureus* takes advantage of it and starts reproducing, draining the resources available for other bacteria.

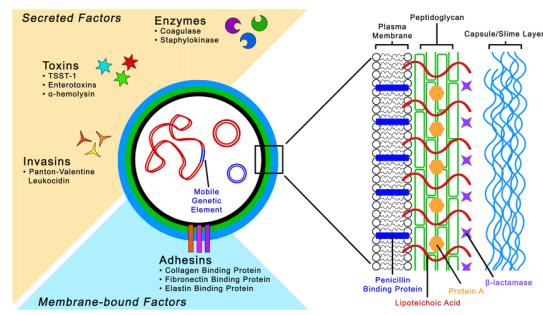


Figure 2.1: Parts of *Staphylococcus aureus*[5].

§2.3 The enemy's attacks

Staphylococcus aureus is a species that can cause a handful of different diseases, ranging from, most frequently, skin and respiratory tract infections to infective endocarditis, toxic shock syndrome or osteomyelitis. Several variations of this pathogen exist, with increasing levels of antibiotic resistance: MSSA (*Methicillin-Sensitive Staphylococcus aureus*), having no resistance; MRSA (*Methicillin-Resistant Staphylococcus aureus*); and VRSA (*Vancomycin-Resistant Staphylococcus aureus*), the latter for which no antibiotic concoction that can eradicate the infection is known, and the patients have to use experimental treatments. VISA (*Vancomycin-intermediate Staphylococcus aureus*) is a variation that has medium resistance to vancomycin, being an intermediate step between MRSA and VRSA. Studies have discovered that this genetic factor has been developed by different lineages separately, indicating that there is not a common ancestor of MRSA strains.

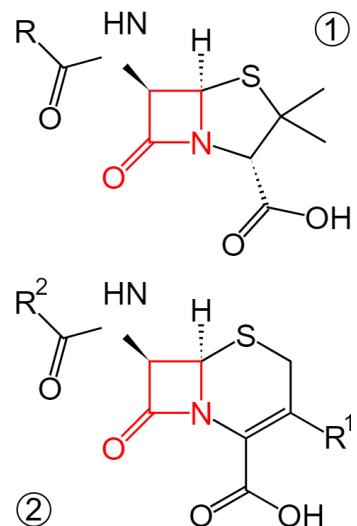
Staphylococcus aureus contains an important quantity of **toxins**, compounds that grant *Staph* most of its pathogenicity. Many of its virulence factors can be described as such. Toxins are usually defined as poisonous substances, which, in our case, means that they have the capacity to mess with the host body directly, without need of a mediating entity. *Staph* has several kinds of toxin in its arsenal: membrane-damaging toxins (which can be receptor-mediated or not), receptor-interfering toxins (which do not damage the membrane), enzymes, and pathway blockers[6].

§2.4 Our weapons

The tools we have at our disposal to fight off this infection fall into two main categories: chemical factors and biological factors.

The chemical factors are drugs, and they depend both in quantity and type on the variation a particular case falls in. It is **extremely important** to find out the level of antibiotic resistance that a specific infection has before administering any antibiotic, as this treatment course will cause side effects such as killing gut bacteria, diminishing defence system capabilities, and increasing the possibility to develop yet more resistant infections. Generally, a large-spectrum antibiotic has an adequate risk-to-benefits ratio of causing the previously mentioned side effects, so they may be used before switching to a more specific (and in some cases even more violent) treatment.

Starting with the treatment to the least resistant strains of *Staphylococcus aureus*, a -lactam antibiotic (such as methicillin, oxacillin, cloxacillin and penicillin) is the weapon of choice to fight against an MSSA infection. This is because this specific chemical part (just a -lactam ring does nothing by itself) has the ability to inhibit cell wall biosynthesis on the bacterial intruder's body. But once the -lactam ring is cut by an enzyme secreted by the bacteria itself, this type of antibiotic suddenly loses effect against them.



That's where vancomycin comes in. It is a type of glycopeptide antibiotic, just like -lactam, and works by blocking the construction of a cell wall, as all of its type do. This treatment is very invasive and only indicated for the treatment of extremely serious, life-threatening infections by Gram-positive bacteria that have shown to be unresponsive to other antibiotics.

Figure 2.2: Organic chemistry structure of penicillin (top) and cephalosporin (bottom). -lactam ring in red. Source: [FileBetalactamAntibiotics2007]

It can be taken as a pill or as an injectable fluid, the latter form proving to be much more effective than the former. This treatment is incompatible with aminoglycosides, a type of antibiotic that inhibits protein synthesis, as it can lead to nephrotoxicity and ototoxicity. Vancomycin can induce internal bleeding, with petechial haemorrhages

on the tongue and bruises on most of the body of the patient. Unfortunately, even with use of vancomycin, *Staphylococcus aureus* can develop resistance. In this case, no other option than using a biological factor is left.

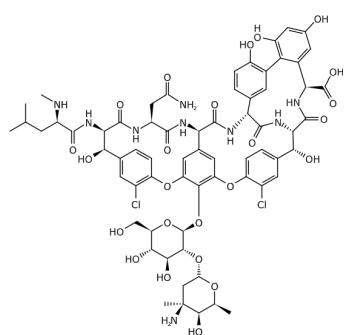


Figure 2.3: Organic chemistry structure of vancomycin. Source: [FileVancomycinSvg2003]

The biological factor is a bacteriophage, called P68. It comes from the *Caudovirales* order, which means that it is a bacteriophage with tail. This treatment is still in testing, but it appears to be effective and lead to low adverse results. If possible, it would be preferable to use bacteriophage therapy (shortened to phage therapy) instead of going for antibiotics, as it can lead to less side effects than antibiotics, as it only attacks a specific bacterium. This means that the infection has to be pinpointed with extreme accuracy. The use of this treatment also negates the risk of bacteria developing antibiotic resistance. It is, however, unclear whether the bacteriophage could mutate into a dangerous strain. This therapy is in clinical research, and may be available soon.[7].

Bacteriophages work in an interesting manner. They work by detecting one very specific bacteria, just like any other virus does with the type of cell they evolved for, then bind to it and inject their genetic material, which then in turn the bacteria considers as its own, inserts it into its own genetic sequence and starts producing the proteins the virus requires, but it doesn't eject them. Once the bacteria is full of phages, a special lytic compound is released which bursts the cell membrane in such a way that it resembles an explosion, but instead of heating up everything in a radius, spreads millions more of bacteriophages, which then bind to other bacteria and the cycle repeats until there's no more bacteria left. The fight from the bacteria point of view consists mostly on trying to outnumber and outreproduce the phages in order to have a chance of survival, even if minimal. There is no known bacteria that shows resistance to phages. That is probably because, unlike the chemical factors, phages can evolve and improve with each generation thanks to natural selection[8].

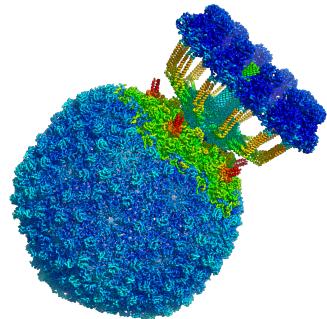


Figure 2.4: P68 structure.
Source: own

3 Experimental design

It is common sense to take a method and try it; if it fails, admit it frankly and try another. But above all, try something.

Anthony Burgess

This Extended Essay has the objective of studying bibliographically the effects of *Staphylococcus aureus* on the human body, as well as the ways humanity has developed to defeat it. Experimentally, it has one main objective, and several secondary ones: answering the research questions posed in the most reliable way I can achieve. Secondly, I want to improve my lab etiquette and fluidity, protocol-making, how I follow protocols in the lab and how I deal with problems that may arise from them, my staining and microscope use, and how I work with limited resources.

The research question I will follow is

"What is the prevalence of Staphylococcus aureus in our school?"

To which my hypothesis is:

"About 30%"

This hypothesis comes from my bibliographical research. I would also like to know the answer to the question

"Is the prevalence of Staphylococcus aureus affected by gender or age?"

to which my hypothesis is

“No“

The variable I will study is the presence or not of the bacterium in question on different subjects, and compare it against their characteristics (such as approximate age and gender). I'll keep constant the culture medium, as well as the culture temperature and humidity.

§3.1 Variables studied

This study studied one dependent variable: the prevalence of *Staphylococcus aureus*, comparing it against two different independent variables: the gender of the subject and the age group of the subject. This will allow me to check for a correlation between these tw

§3.2 Bill of materials

The materials used, as well as the quantities used, can be found in the following table. On the left, laboratory equipment and, on the right, reagents, staining agents, and consumables used:

Qty	Material/consumable	Qty	Reagent
x80	Sterile cotton swabs	~30mL	Bleach
x1	Kolle handle	~10mL	Methyl violet
x1	Optic microscope	~10mL	Iodine
x1	Binocular magnifier	~10mL	Alcoholic safranin
x1	Dissection tray	~10mL	Methanol
x1	Bunsen burner	<1mL	Ether
x1	Lab coat	x40	Agar MSA plates
x1	Lab goggles	>1L	Ringer solution (9% saline)
x8	Non-powdered gloves		
x10	Slides and slide covers		

§3.3 Biosecurity and risk mitigation

Staph is considered a Biosecurity Level 2 bacterium[9]. This means that it is associated with a human disease that can pose a moderate human health hazard. In a laboratory where such individua are handled, normal lab etiquette should be followed, as well as avoiding splashes or aerosols, adhering biohazard warning signs present on all material used, and proper surfaces and material disinfection via the use of autoclave.

The risks associated with this bacterium were assessed following the 2020 Biosafety Manual published by the WHO, and proper security measures were followed at all times when handling biohazardous material. No incidents occurred during the research[2].

4 Physical experimentation

A scientist in his laboratory is not a mere technician: he is also a child confronting natural phenomena that impress him as though they were fairy tales.

Marie Curie

§4.1 Description

This experiment is designed to detect and evaluate the prevalence of *Staphylococcus aureus* in a sample of students from our school. The process used involves extracting a sample from underneath a subject's nails by swabbing, cultivating that sample, and then observing the results of said culture to determine the presence or not of *Staphylococcus aureus* as part of the subject's resident bacterial flora. Each sampling iteration of the process took less than two minutes to complete. However, all the safety measures and actions taken need more time to be taken care of properly; as well as taking into account the fact that cultivating is not a task that can be done in just a day, often needing two to three to fully develop.

§4.2 Protocol followed

The protocol followed was designed based on a similar protocol used in many university laboratories[10], modified to fit the needs of this research paper. This protocol underwent 10 different revisions. It dictates the following steps:

- 1) Set up the work area; the Bunsen burner should be turned on in such a way that it can cover an acceptable surface to work. Turn it on and try not to break the sterile field.
- 2) Prepare yourself for the experimentation: wash your hands, proper PPE. Wash your hands again (with gloves on).
- 3) Divide each Petri dish in 2 parts. A ruler should be used for this part. Get your subject to wash their hands and observe them. If the nails are extremely short, it may be worth it to take the sample nasally. If the hands don't seem clean enough, teach them proper hand washing techniques.
- 4) Note down their information, crack open a sterile swab pack, dip one of the swabs in Ringer solution and swab under their nails or nose. Then, populate the dish with this sample following the zig-zag method for one of the halves of the dish.
- 5) Incubate for 32-48h and observe the results.
- 6) Observe the bacteria under a microscope after a GRAM staining.

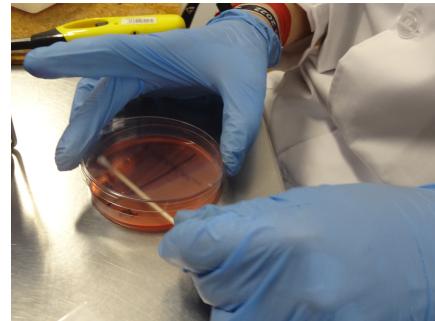


Figure 4.1: Photograph of me populating a Petri dish.
Source: own

§4.3 Results and analysis

The results obtained can be found in the following raw data table:

Group	Plate	Result	Group	Plate	Result
1BAT A	E1	Positive	FAM	A5	Positive
4ESO B	B12	Negative	FAM	A9	Unknown
1BAT A	C2	Negative	FAM	A10	Unknown
PROF	B4	Positive	FAM	A11	Negative
PROF	F4	Negative	FAM	A12	Positive
1BAT A	E2	Negative	PROF	B1	Positive
1BAT A	E5	Negative	PROF	B3	Positive
1BAT A	E7	Positive	4ESO B	B7	Negative
1BAT A	E6	Positive	4ESO B	B8	Negative
PROF	C1	Negative	4ESO B	B9	Negative
PROF	B2	Negative	4ESO D	B10	Negative
1BAT A	E8	Positive	4ESO D	B11	Positive
1BAT A	E4	Positive	4ESO D	B13	Positive
FAM	D1	Positive	4ESO D	B14	Negative
1BAT A	E3	Negative	PROF	C3	Positive
PROF	B5	Positive	FAM	D2	Negative
FAM	A1	Negative	PROFJ	F1	Positive
FAM	A2	Positive	PROFJ	F2	Positive
FAM	A3	Negative	1 Bat A	F3	Negative
FAM	A4	Positive	1 Bat A	E9	Negative
FAM	A6	Negative	1 Bat A	E10	Negative
FAM	A7	Negative	1BAT A	E11	Negative
FAM	A8	Positive	1BAT A	E12	Positive
			PROF	A9	Positive

The data was then recounted and graphed into the following pie chart:

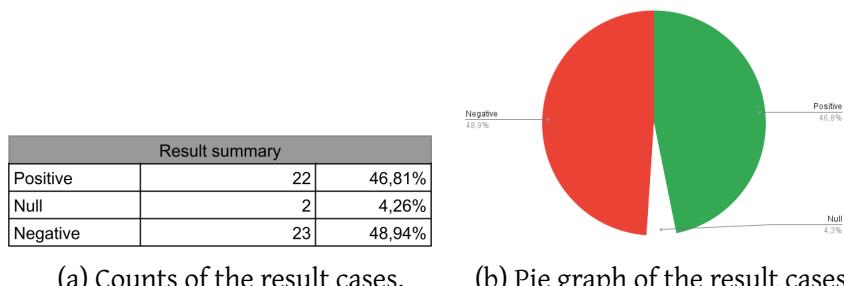
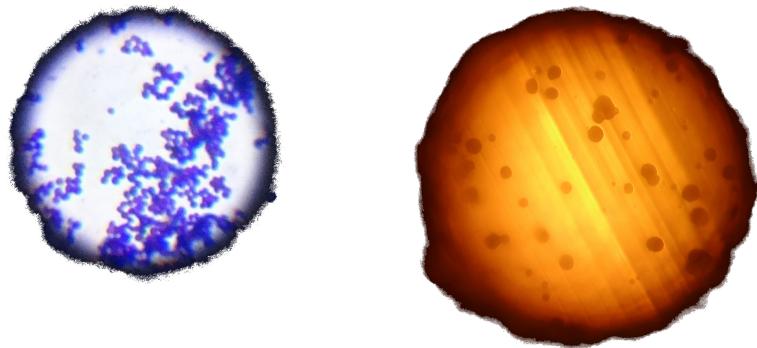


Figure 4.2: Data processed from results

As we can see, almost 50% of the samples taken tested positive for *Staphylococcus aureus*, compared to the expected 30%[11]. We can, however, see in the UK's Public Health bactaeremia data that Staph infections have been on the rise lately, so it may not be a case of wrong data[12]. On top of that, both of the experts I emailed, to see if they had also seen an uptick in cases, also found their experiments resulting in a higher prevalence than usual of this bacterium, and found cases that were once negative but recently turned positive. Most of these results were not only confirmed by the highly-specific detection of the MSA plate, but also by taking the morphological observation into account, both of the colonies and microscopically.

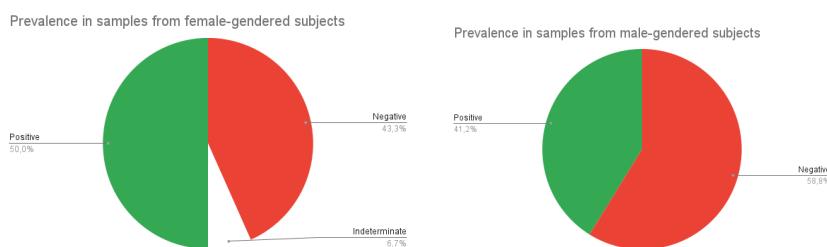
There may be several reasons for the infection rate and thus natural prevalence to be increasing. One of them could be that since antibiotic abuse is growing with each passing year, the usual resident microbiota is getting killed, leaving more resources for Staph to thrive in that environment. To confirm this theory, we will look at the infection rates of a country that is facing extreme antibiotic abuse (the United States of America) and compare it to another that is controlling their antibiotics a bit better (the United Kingdom). The former have seen a 210% increase in *Staphylococcus aureus* cases since 2006. However, superfluous antibiotic prescriptions have increased by barely 1%[13]. In the United Kingdom, they have seen a 160% increase in *Staphylococcus aureus* infections[12], and their superfluous antibiotic prescriptions have gone down by 20%. Even though this is very little data to extract conclusions from, there may be a correlation between these two factors.



(a) *Staphylococcus aureus* as seen below the microscope. x4000, GRAM
 (b) Colonies of *Staphylococcus aureus* seen under a magnifying glass

Figure 4.3: Photographies of the results, as collected from my own experimentation (own data).

Let's compare the prevalence among different groups of subjects, starting with their gender.



(a) Counts of the result cases in female-gendered individuals (b) Counts of the result cases in male-gendered individuals

Figure 4.4: Data processed from results

As we can observe, there is a 10% difference in prevalence between these two genders. In my opinion, this is due to a small sample size. As we can see, the first graph, which houses the samples from subjects who identify themselves as female, has a third type of

result, which the other graph, which houses the samples from subjects who self-identify as male, does not. This is simply due to a problem with the plates, however, it may affect the final result. I believe there to not be any significant difference between the two analized genders.

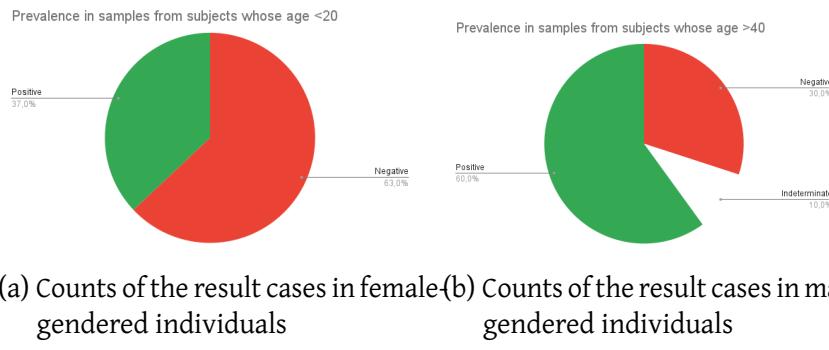


Figure 4.5: Data processed from results

Only two age groups were studied, due to the fact that the samples fell mostly into one of these two categories. We can see that the samples from a subject older than 40 were twice as likely to test positive for *Staphylococcus aureus* than those from subjects younger than 20. This may again be due to a small sample size. However, it may also be possible that the possibility of hosting this bacterium increases with age. In order to confirm this theory, more studies should be done, with a greater age gradient, as well as many more samples.

5 Conclusions

Our reliance on the validity of a scientific conclusion depends ultimately on a judgement of coherence; and as there can exist no strict criterion for coherence, our judgement of it must always remain a qualitative, non-formal, tacit, personal judgement.

Michael Polanyi

§5.1 Bibliographic conclusions

While *Staphylococcus aureus* is a dangerous bacterium given the right conditions However, most times, the immune system can get rid of it before it becomes too large of a problem. However, in some cases, when the entire body gets infected and the infection stops being localized, then that's when there is a problem. There are several strains of *Staphylococcus aureus*, classified by their resistance to antibiotics: MSSA (sensitive to methicillin), MRSA (resistant to methicillin), VISA (intermediate resistance to vancomycin) and VRSA (resistant to vancomycin). While there is no antibiotic that can deal with VRSA, an alternative in the form of a bacteriophage virus, P-68, of the order of the *Caudovirales*

§5.2 Experimental conclusions

This study has concluded that the prevalence of *Staphylococcus aureus* in our high school is 48,8%, one and a half times the expected results. As explored previously, this could mostly be due to climate change or antibiotic abuse, however there may also be other

reasons for why this is happening. Our initial hypothesis, which was that the prevalence of epithelial *Staphylococcus aureus* in school would be at around 30% was found out to be false. Instead, the experimentation found a prevalence of the bacteria being 46,5%. This value, 1,5 times larger than the one expected. However, it probably did not come from experimental error, as the procedure was followed rigorously, and the risk of contamination was mitigated to levels with which we could confidently say that no plates were subjects of cross-contamination between batches.

The analysis of prevalence studied as a variable dependent to the subject's gender seemed to bear no different conclusions to the ones stated above. However, if studied according to the subject's age, we could see that the group comprising the samples from the older subjects were twice as likely to test positive than those from the younger subjects. Seemingly, my initial hypothesis for the second question was wrong.

§5.3 Strengths and weaknesses

This research was not without its strengths, but neither was it without its weaknesses.

Strengths The protocol was adapted fairly well to the environment it was run in, and no incidents took place during the realization of the experimentation. The cost of the experimentation was relatively cheap, taking into account that reagents in microbiology can quickly get expensive. Reliability was also high, and the questions were answered, hypotheses verified and refused.

Weaknesses While the Agar plates used were definitely adequate for the purpose they were used for, a much more appropriate growth medium called Baird-Parker (BP) could've been used. A much more adequate and comfortable lab environment would also have been a very welcomed improvement. A much larger sample size could also have helped in giving much more accurate and precise answers to the questions asked.

§5.4 Possible improvements

This research could've been improved by running an antibiogram on the samples, thus checking for antibiotic resistance. While this is fairly safe if adequate protections are taken, it is yet another point that could fail and result in a biosafety incident.

It could have also been improved by obtaining even a larger sample of the population, in order to get an even more significant result. The bacteria could have been sequenced, allowing us to trace back the bacterium one by one, comparing it to locations where a similar strain had been found, tracing back its evolution and possible path followed around the world.

Another interesting factor to be looked at could be the familial relationship between subjects that tested positive. It may be possible that there is some genetic character that causes members of a same family to have a predisposition of having *Staphylococcus aureus* under their nails, as compared to subjects not from the same family.

If given more time and resources, I would also have liked to look at the relationship between prevalence, age and gender with much more detail. In case I ever get to improve on my research, this will probably be the factor that I study: the relationship between the age of the subject and their likeliness to test positive to *Staphylococcus aureus*

Works referenced

- [1] *What Is GDPR, the EU's New Data Protection Law?* <https://gdpr.eu/what-is-gdpr/>. Nov. 2018.
- [2] World Health Organization. *Laboratory Biosafety Manual*. 4th ed. Laboratory Biosafety Manual, Fourth Edition and Associated Monographs; Geneva: World Health Organization, 2020. Chap. The Portuguese version is published by PAHO: <https://iris.paho.org/handle/10665.2/54521>. ISBN: 978-92-4-001131-1.
- [3] Patrick R Murray, Ken S Rosenthal and Michael A Pfaller. *Microbiología médica*. Barcelona: Elsevier, 2013. ISBN: 978-84-9022-411-3.
- [4] *Dorlands Medical Dictionary:Disease*. https://web.archive.org/web/20100411075617/http://www.mercksource.com/pp/us/cns/cns_hl_dorlands_split.jsp?pg=/ppdocs/us/common/dorlands/dorland/three/000030493.htm. Apr. 2010.
- [5] Eric F. Kong, Jennifer K. Johnson and Mary Ann Jabra-Rizk. 'Community-Associated Methicillin-Resistant Staphylococcus Aureus: An Enemy amidst Us'. In: *PLOS Pathogens* 12.10 (Oct. 2016). Ed. by John M Leong, e1005837. ISSN: 1553-7374. DOI: [10/gdm3tb](https://doi.org/10/gdm3tb).
- [6] Cin Kong, Hui-min Neoh and Sheila Nathan. 'Targeting Staphylococcus Aureus Toxins: A Potential Form of Anti-Virulence Therapy'. In: *Toxins* 8.3 (Mar. 2016), E72. ISSN: 2072-6651. DOI: [10/f8tnpx](https://doi.org/10/f8tnpx).
- [7] Kaixiang Zhou et al. 'A Review on Nanosystems as an Effective Approach against Infections of Staphylococcus Aureus'. In: *International Journal of Nanomedicine* 13 (2018), pp. 7333–7347. ISSN: 1178-2013. DOI: [10/gfp2z2](https://doi.org/10/gfp2z2).
- [8] Dominik Hrebík et al. 'Structure and Genome Ejection Mechanism of Staphylococcus Aureus Phage P68'. In: *Science Advances* 5.10 (Oct. 2019), eaaw7414. ISSN: 2375-2548. DOI: [10/gm742z](https://doi.org/10/gm742z).
- [9] Gordon Y. C. Cheung, Justin S. Bae and Michael Otto. 'Pathogenicity and Virulence of Staphylococcus Aureus'. In: *Virulence* 12.1 (Dec. 2021), pp. 547–569. ISSN: 2150-5608. DOI: [10/gm3xqz](https://doi.org/10/gm3xqz).

- [10] William M. O'Leary, ed. *Practical Handbook of Microbiology*. Boca Raton, Fla: CRC Press, 1989. ISBN: 978-0-8493-3704-8.
- [11] *Staphylococcus Aureus in Healthcare Settings | HAI | CDC*. <https://www.cdc.gov/hai/organisms/staph.html>. Dec. 2020.
- [12] Public Health England. *MSSA Bacteraemia: Annual Data*. <https://www.gov.uk/government/statistics/mssa-bacteraemia-annual-data>. Sept. 2021.
- [13] James Baggs et al. 'Estimating National Trends in Inpatient Antibiotic Use Among US Hospitals From 2006 to 2012'. In: *JAMA Internal Medicine* 176.11 (Nov. 2016), pp. 1639–1648. ISSN: 2168-6106. DOI: [10/ggqsvf](https://doi.org/10/ggqsvf).

I

Appendix

Annex 1 – Informed consent given to subjects before sampling

CONSENT TO BE PART OF A RESEARCH STUDY

1. KEY INFORMATION ABOUT THE RESEARCHERS AND THIS STUDY

Study title: A study on the affection and effects of Staphylococcus Aureus



You are invited to take part in a research study. This form contains information that will help you decide whether to join the study.

If you choose to participate, you will be asked to go to the Biology Laboratory PN22, wash profusely your hands and have a sample taken from the subungual tissue. This poses no risk andd will take around 2 to 7 minutes. The results will be delivered to you in 24-48 hours, in paper form. There are no other direct benefits.

Taking part in this research project is voluntary. You do not have to participate and you can stop at any time. Please take time to read this entire form and ask questions before deciding whether to take part in this research project.

2. PURPOSE OF THIS STUDY

The purpose of this study is to evaluate the percentage of students whose reseident bacterioflora include *Staphylococcus aureus*, as well as a genomic analysis of a random one of the positive samples.

We may use the subungual tissue collected for this study for whole bacterial genome sequencing which involves mapping all of the bacteria DNA to screen for MRSA and MSSA.

3. WHO CAN PARTICIPATE IN THE STUDY

3.1 Who can take part in this study? There is no application criteria.

3.2 How many people are expected to take part in this study? About 40-60 people are expected to take part in this study.

4. INFORMATION ABOUT STUDY PARTICIPATION

4.1 What will happen to me in this study?

- You will be called to the Biology Laboratory
- You will be asked to follow simple instructions to wash your hands
- A sample from below your nails will be taken and cultured
- If positive, your sample may be chosen for sequencing.
- You will be given all your results 24-48h after sampling in paper form.

The process can be found in detail at dx.doi.org/10.17504/protocols.io.81wgb6pk1lpk/v6

4.2 How much of my time will be needed to take part in this study? This will take one day, 5-10 minutes total maximum.

4.3 If I decide not to take part in this study, what other options do I have?
Leave and not get your results.

5. INFORMATION ABOUT STUDY RISKS AND BENEFITS

5.1 What risks will I face by taking part in the study? What will the researchers do to protect me against these risks?

There are no known risks.

5.2 How could I benefit if I take part in this study? How could others benefit?

You may not receive any personal benefits from being in this study. However, others may benefit from the knowledge gained from this study. You will receive your results.

5.2.1 Will the researchers provide information to me about what they learn from analyzing my [type of biospecimen]? We may learn things about your health as part of the research. If this happens, this information will be provided to you. [Insert a description of the types of research results that may be returned, under what circumstances participants will be provided research results, and how participants will be notified.] You may need to meet with professionals with expertise to help you learn more about your research results. The study team/study will not cover the costs of any follow-up consultations or actions.

5.3 Will the researchers tell me if they learn of new information that could change my willingness to stay in this study? Yes, the researchers will tell you if they learn of important new information that may change your willingness to stay in this study.

6. ENDING THE STUDY

6.1 If I want to stop participating in the study, what should I do?

You are free to leave the study at any time. If you leave the study before it is finished, there will be no penalty to you. You will not lose any benefits to which you may otherwise be entitled. If you decide to leave the study before it is finished, please tell one of the persons listed in Section 9 "Contact Information". If you choose to tell the researchers why you are leaving the study, your reasons may be kept as part of the study record. The researchers will keep the information [and type of biospecimen] collected about you for the research unless you ask us to remove the information from our records and destroy the [type of biospecimen]. If the researchers have already used your information in a research analysis, it will not be possible to remove your information.

7. FINANCIAL INFORMATION

No money will be transferred.

8. PROTECTING AND SHARING RESEARCH INFORMATION [AND BIOSPECIMENS]

8.1 How will the researchers protect my information? Following GDPR regulations.

8.2 Who will have access to my research records?

There are reasons why information about you may be used or seen by the researchers or others during or after this study. Examples include:

- University, government officials, study sponsors or funders, auditors, and/or the Institutional Review Board (IRB) may need the information to make sure that the study is done in a safe and proper manner.

8.3 What will happen to the information and/or biospecimens collected in this study?

We will keep the information and/or biospecimens we collect about you during the research, [including information we learn from analyzing your sample, for future research projects and for study recordkeeping. Your name and other information that can directly identify you will be stored securely and separately from the research information we collected from you..

The results of this study could be published in an article or presentation, but will not include any information that would let others know who you are.

8.4 Will my information and/or biospecimens be used for future research or shared with others?

We may use or share your research information and/or biospecimen for future research studies. If we share your information and/or biospecimen with other researchers it will be de-identified, which means that it will not contain your name or other information that can directly identify you. This research may be similar to this study or completely different. We will not ask for your additional informed consent for these studies.

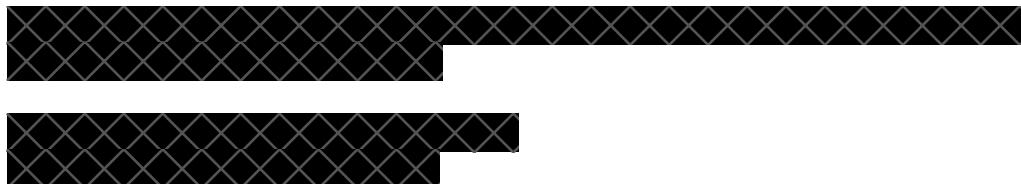
We would like to share your identifiable information or biospecimen with other researchers for future research. We will ask for your consent to do so at the end of this consent document. You can be a part of this current research project without agreeing to this future use of your identifiable information or biospecimen.

9. CONTACT INFORMATION

Who can I contact about this study?

Please contact the researchers listed below to:

- Obtain more information about the study
- Ask a question about the study procedures
- Report an illness, injury, or other problem (you may also need to tell your regular doctors)
- Leave the study before it is finished
- Express a concern about the study



Annex 2 – GDPR notice given to subjects before sampling

GDPR NOTICE

You are receiving this notice in connection with your participation in the following research study:

Title of Study: A study on the affectation and effects of Staphylococcus Aureus

Principal Investigator: [REDACTED]

The above-named research study involves the collection of *sensitive personal data* that can identify you. The General Data Protection Regulation ("GDPR") requires researchers to provide this notice to you when we collect and use research data about people located within the European Union (EU) or the European Economic Area (EEA). This notice outlines what personal data we will collect, how we intend to use and protect this information, and your rights with respect to your personal data for purposes of GDPR.

NOTE: The GDPR may apply to *personal data* that you provide while physically located in the EU/EEA. It does not apply to information provided while located outside of the EU/EEA (e.g., while in the United States). GDPR data protection requirements do not apply to your personal data that is rendered anonymous such that you are not identifiable or can no longer be identified.

Personal data – what we will collect

As part of this research study, we will create and obtain information related to your participation in the study from you so we can conduct this research. Research study data will include: contact information and physiological data that arises from the test, which will include the presence or not of bacteria and the concentration of it.

How we will use your Personal Data

The personal data you provide will be used for the following purposes:

- To fulfill study objectives as described within the Study Informed Consent Form
- To provide study compensation and complying with compensation-related reporting requirements
- To comply with legal and regulatory requirements, including requirements to share data with regulatory agencies overseeing the research
- To confirm proper conduct of the study and research integrity

Your personal data may be transferred to the United States in condition of storage. The United States does not have the same laws to protect your personal data as in the EU/EEA. However, we are committed to protecting the confidentiality of the personal data you give us. The *Study Informed Consent form* further describes the protections in place to protect the confidentiality of your personal data. Transfer and use of your personal data is on the basis of your consent.

Retention of your personal data

We may retain your personal data for as long as necessary to fulfill the objectives of

the research and to ensure the integrity of the research. We will delete your personal data when it is no longer needed for the study or if you withdraw your consent provided such deletion does not render impossible or seriously impair the achievement of the objectives of the research project. However, your information will be retained as necessary to comply with legal or regulatory requirements. Your data will be anonymized as soon as the results of your test have been sent to you by using a unique randomized identifier. No non-anonymized data will be used for future studies.

Your rights with respect to your personal data

If you participate in this study within the EU/EEA the GDPR affords you certain rights with respect to your personal data, including the right to:

- Access, correct, withdraw, or delete your personal data; however, the research team may need to keep your personal data as long as it is necessary to achieve the purpose of this research;
- Restrict the types of activities the research team can do with your personal data;
- Object to using your personal data for specific types of activities; or
- Withdraw your consent to use your personal data for the purposes outlined in the *Study Informed Consent form* and in this document. (However, this withdrawal will only apply to new personal data not yet collected or created. Personal data already collected or created may continue to be used as outlined in the *Study Informed Consent form* and this document.)

To exercise your rights, please use the contact information below to submit a request. When you submit a request, please indicate your name, the name of this project, your reasons for making the request, if necessary, and other details you think will be useful for us to comply with your request.

Where to address your questions or concerns about your personal data

If you want to make a request relating to the rights listed above or if you have any concerns about how your personal data is being handled, please contact:



Your Consent

Your consent is entirely voluntary, but declining to provide consent may impede your ability to participate in this research project.

By clicking below, you indicate that you have read and understood how your personal data will be processed, your related rights, and that you consent to the processing of your data as provided in this document. In addition, you acknowledge

that this information was explained to you, your questions have been answered, and that you wish to continue participating in the study. If any new questions arise, you can contact the research team using the information provided above.

You may print a copy of this form for your files.

Name:

I acknowledge that this new information was explained to me, my questions have been answered to my satisfaction, and I wish to participate in this study.

Annex 3 – Bibliography consulted (but not referenced)

- [1] G. Verger, Ll Carbó and Fundació Dr Antoni Esteve. *Problemas que se plantean en el tratamiento de infecciones graves por S. Aureus* / [editores]: G. Verger, Ll. Carbó. Barcelona, 1986.
- [2] William M. O'Leary, ed. *Practical Handbook of Microbiology*. Boca Raton, Fla: CRC Press, 1989. ISBN: 978-0-8493-3704-8.
- [3] Alan J. Lacey, ed. *Light Microscopy in Biology: A Practical Approach*. 2. ed. The Practical Approach Series 195. Oxford: Oxford Univ. Press, 1999. ISBN: 978-0-19-963669-3 978-0-19-963670-9.
- [4] Anthony J. F. Griffiths, ed. *Modern Genetic Analysis*. 3rd print. New York, NY: W. H. Freeman, 2000. ISBN: 978-0-7167-3597-7 978-0-7167-3118-4 978-0-7167-3347-8.
- [5] Anna Claret i Coma. ‘Resistència antibòtica en les poblacions de lactobacils, estafilococs i entreococs aïllats de productes lleugerament fermentats.’ Projecte/Treball de Final de Carrera. Girona: Universitat de Girona, May 2004.
- [6] David W. Mount. *Bioinformatics: Sequence and Genome Analysis*. 2nd ed. Cold Spring Harbor, N.Y: Cold Spring Harbor Laboratory Press, 2004. ISBN: 978-0-87969-687-0 978-0-87969-712-9.
- [7] Matthew J. Kuehnert et al. ‘Prevalence of Staphylococcus Aureus Nasal Colonization in the United States, 2001-2002’. In: *The Journal of Infectious Diseases* 193.2 (Jan. 2006), pp. 172–179. ISSN: 0022-1899. DOI: [10/c8985p](https://doi.org/10/c8985p).
- [8] Fvasconcellos WikMedia user. *File:Beta-lactam Antibiotics Example 1.Svg - Wikipedia*. https://commons.wikimedia.org/wiki/File:Beta-lactam_antibiotics_example_1.svg. Oct. 2007.
- [9] BAIRD-PARKER Agar (*Staphylococcus Selective Agar Base Acc. to BAIRD-PARKER*). https://web.archive.org/web/20080501041929/http://www.emdchemicals.com/analytics/Micro_Manual/TEDIS-data/prods/1_05406_0500.html. May 2008.
- [10] BartVL71 WikMedia user. *File:Vancomycin.Svg - Wikipedia*. <https://commons.wikimedia.org/wiki/File:Vancomycin.svg>. Oct. 2008.
- [11] Dong Xu and Yang Zhang. ‘Generating Triangulated Macromolecular Surfaces by Euclidean Distance Transform’. In: *PLoS ONE* 4.12 (Dec. 2009). Ed. by Markus J. Buehler, e8140. ISSN: 1932-6203. DOI: [10/d6tf9f](https://doi.org/10/d6tf9f).
- [12] Dorlands Medical Dictionary:Disease. https://web.archive.org/web/20100411075617/http://www.mercksource.com/pp/us/cns/cns_hl_dorlands_split.jsp?pg=/ppdocs/us/common/dorlands/dorland/three/000030493.htm. Apr. 2010.
- [13] Carlos Gamazo, Ramón Díaz and Ignacio López-Goñi. *Manual práctico de microbiología*. Barcelona: Elsevier Masson, 2010. ISBN: 978-84-458-1519-9.
- [14] Global Warming. <https://earthobservatory.nasa.gov/features/GlobalWarming/page2.php>. Text.Article. June 2010.

- [15] John T. Moore and Richard Langley. *Biochemistry for Dummies*. 2nd ed. –For Dummies. Hoboken, NJ: Wiley Pub, 2011. ISBN: 978-1-118-02174-3.
- [16] Gloria Inés Puerta Quintero. ‘Fundamentos Del Proceso de Fermentación En El Beneficio Del Café’. In: *Avances técnicos Cenicafe* (2012). ISSN: 0120-0178.
- [17] Patrick R Murray, Ken S Rosenthal and Michael A Pfaller. *Microbiología médica*. Barcelona: Elsevier, 2013. ISBN: 978-84-9022-411-3.
- [18] James Baggs et al. ‘Estimating National Trends in Inpatient Antibiotic Use Among US Hospitals From 2006 to 2012’. In: *JAMA Internal Medicine* 176.11 (Nov. 2016), pp. 1639–1648. ISSN: 2168-6106. DOI: [10/ggqsvf](https://doi.org/10/ggqsvf).
- [19] NASA’s GMS. *GMS: Annual Global Temperature, 1880-2015*. <https://svs.gsfc.nasa.gov/12133>. Jan. 2016.
- [20] Cin Kong, Hui-min Neoh and Sheila Nathan. ‘Targeting Staphylococcus Aureus Toxins: A Potential Form of Anti-Virulence Therapy’. In: *Toxins* 8.3 (Mar. 2016), E72. ISSN: 2072-6651. DOI: [10/f8tnpx](https://doi.org/10/f8tnpx).
- [21] Eric F. Kong, Jennifer K. Johnson and Mary Ann Jabra-Rizk. ‘Community-Associated Methicillin-Resistant Staphylococcus Aureus: An Enemy amidst Us’. In: *PLOS Pathogens* 12.10 (Oct. 2016). Ed. by John M Leong, e1005837. ISSN: 1553-7374. DOI: [10/gdm3tb](https://doi.org/10/gdm3tb).
- [22] Tzu-Sen Yang et al. ‘Disinfection Effects of Undoped and Silver-Doped Ceria Powders of Nanometer Crystallite Size’. In: *International Journal of Nanomedicine* 11 (June 2016), p. 2531. DOI: [10/f8p99f](https://doi.org/10/f8p99f).
- [23] Professor Dave Explains. *Viruses: Molecular Hijackers*. Oct. 2017.
- [24] Xiaopeng Zhang, Xiaomei Hu and Xiancai Rao. ‘Apoptosis Induced by Staphylococcus Aureus Toxins’. In: *Microbiological Research* 205 (Dec. 2017), pp. 19–24. ISSN: 1618-0623. DOI: [10/gcf5pm](https://doi.org/10/gcf5pm).
- [25] SAHIL BATRA. *Biochemical Tests for Staphylococcus Aureus / Bacteriology Notes*. <https://paramedicsworld.com/staphylococcus-aureus/biochemical-tests-staphylococcus-aureus/medical-paramedical-studynotes>. Sept. 2018.
- [26] SAHIL BATRA. *Biochemical Tests For Streptococcus Pneumoniae / Bacteriology Notes*. <https://paramedicsworld.com/streptococcus-pneumoniae-pneumococcus/biochemical-tests-for-streptococcus-pneumoniae/medical-paramedical-studynotes>. Sept. 2018.
- [27] TED. *How a Long-Forgotten Virus Could Help Us Solve the Antibiotics Crisis / Alexander Belcredi*. 2018.
- [28] *What Is GDPR, the EU’s New Data Protection Law?* <https://gdpr.eu/what-is-gdpr/>. Nov. 2018.
- [29] Kaixiang Zhou et al. ‘A Review on Nanosystems as an Effective Approach against Infections of Staphylococcus Aureus’. In: *International Journal of Nanomedicine* 13 (2018), pp. 7333–7347. ISSN: 1178-2013. DOI: [10/gfp2z2](https://doi.org/10/gfp2z2).

- [30] Shayla Hesse and Sankar Adhya. ‘Phage Therapy in the Twenty-First Century: Facing the Decline of the Antibiotic Era; Is It Finally Time for the Age of the Phage?’ In: *Annual Review of Microbiology* 73.1 (Sept. 2019), pp. 155–174. ISSN: 0066-4227, 1545-3251. DOI: [10/gqwcss](https://doi.org/10/gqwcss).
- [31] Dominik Hrebík et al. ‘Structure and Genome Ejection Mechanism of *Staphylococcus Aureus* Phage P68’. In: *Science Advances* 5.10 (Oct. 2019), eaaw7414. ISSN: 2375-2548. DOI: [10/gm742z](https://doi.org/10/gm742z).
- [32] Dominik Hrebík et al. ‘Structure and Genome Ejection Mechanism of *Staphylococcus Aureus* Phage P68’. In: *Science Advances* 5.10 (Oct. 2019), eaaw7414. ISSN: 2375-2548. DOI: [10/gm742z](https://doi.org/10/gm742z).
- [33] Aleksandra Petrovic Fabijan et al. ‘Safety of Bacteriophage Therapy in Severe *Staphylococcus Aureus* Infection’. In: *Nature Microbiology* 5.3 (Mar. 2020), pp. 465–472. ISSN: 2058-5276. DOI: [10/gqbj7f](https://doi.org/10/gqbj7f).
- [34] *Staphylococcus Aureus in Healthcare Settings | HAI | CDC*. <https://www.cdc.gov/hai/organisms/staph.html>. Dec. 2020.
- [35] World Health Organization. *Laboratory Biosafety Manual*. 4th ed. Laboratory Biosafety Manual, Fourth Edition and Associated Monographs; Geneva: World Health Organization, 2020. Chap. The Portuguese version is published by PAHO: <https://iris.paho.org/handle/10665.2/54521>. ISBN: 978-92-4-001131-1.
- [36] 74 Asamblea Mundial de la Salud. *Mejora de la bioseguridad en los laboratorios*. Tech. rep. Ginebra: Organización Mundial de la Salud, 2021. Chap. 7 p.
- [37] 74 Assemblée mondiale de la Santé. *Renforcement de la sécurité biologique en laboratoire*. Tech. rep. Genève: Organisation mondiale de la Santé, 2021. Chap. 7 p.
- [38] Gordon Y. C. Cheung, Justin S. Bae and Michael Otto. ‘Pathogenicity and Virulence of *Staphylococcus Aureus*’. In: *Virulence* 12.1 (Dec. 2021), pp. 547–569. ISSN: 2150-5608. DOI: [10/gm3xqz](https://doi.org/10/gm3xqz).
- [39] Public Health England. *MSSA Bacteraemia: Annual Data*. <https://www.gov.uk/government/statistics/mssa-bacteraemia-annual-data>. Sept. 2021.
- [40] John Jumper et al. ‘Highly Accurate Protein Structure Prediction with AlphaFold’. In: *Nature* 596.7873 (2021), pp. 583–589. DOI: [10/gk7nfp](https://doi.org/10/gk7nfp).
- [41] Organisation mondiale de la Santé. *Renforcer la résilience du système de santé pour instaurer la couverture sanitaire universelle et la sécurité sanitaire pendant et après la COVID-19 : exposé de la position de l'OMS*. Tech. rep. Genève: Organisation mondiale de la Santé, 2021. Chap. xii, 39 p.
- [42] 74 World Health Assembly. *Enhancement of Laboratory Biosafety*. Tech. rep. Geneva: World Health Organization, 2021. Chap. 6 p.

- [43] World Health Organization. *Guidance Framework for Testing Genetically Modified Mosquitoes*. 2nd ed. Geneva: World Health Organization, 2021. Chap. xxvi, 165 p. ISBN: 978-92-4-002523-3.
- [44] World Health Organization = Organisation mondiale de la Santé. ‘Case Report of Laboratory-Acquired Vaccinia Virus Infection in India Cas d’infection En Laboratoire Par Le Virus de La Vaccine En Inde’. In: *Weekly Epidemiological Record = Relevé épidémiologique hebdomadaire* 96.05/06 (Feb. 2021), pp. 33–39.
- [45] World Health Organization = Organisation mondiale de la Santé. ‘Weekly Epidemiological Record, 2020, Vol. 96, 05/06 [Full Issue]’. In: *Weekly Epidemiological Record = Relevé épidémiologique hebdomadaire* 96.05/06 (Feb. 2021), pp. 33–44.
- [46] Anaïs Eskenazi et al. ‘Combination of Pre-Adapted Bacteriophage Therapy and Antibiotics for Treatment of Fracture-Related Infection Due to Pandrug-Resistant Klebsiella Pneumoniae’. In: *Nature Communications* 13.1 (Dec. 2022), p. 302. ISSN: 2041-1723. DOI: [10/hdbt](https://doi.org/10/hdbt).
- [47] *Fig. 2. Effect of Temperature on the Growth of S. Aureus*. https://www.researchgate.net/figure/Effect-of-temperature-on-the-growth-of-S-aureus_fig6_266137314. Sept. 2022.
- [48] M.R. KFL082 and Olga Sánchez. ‘Staphilococcus Aureus Sampling V10’. In: *protocols.io* 8 (Sept. 2022). DOI: [10/gqwcst](https://doi.org/10/gqwcst).
- [49] Organisation mondiale de la Santé. *Analyses en laboratoire pour la détection du virus de la variole du singe (orthopoxvirose simienne) : orientations provisoires*, 23 mai 2022. Tech. rep. Genève: Organisation mondiale de la Santé, 2022. Chap. 7 p.
- [50] Organización Mundial de la Salud. *Pruebas de laboratorio para el virus de la viruela símica: orientaciones provisionales*, 23 de mayo de 2022. Tech. rep. Ginebra: Organización Mundial de la Salud, 2022. Chap. 7 p.
- [51] *Plasma Membrane (Cell Membrane)*. <https://www.genome.gov/genetics-glossary/Plasma-Membrane>. Sept. 2022.
- [52] World Health Organization. *Global Guidance Framework for the Responsible Use of the Life Sciences: Mitigating Biorisks and Governing Dual-Use Research*. Geneva: World Health Organization, 2022. ISBN: 978-92-4-005610-7.
- [53] World Health Organization. *Joint External Evaluation Tool: International Health Regulations (2005)*. 3rd ed. Geneva: World Health Organization, 2022. Chap. v, 132 p. ISBN: 978-92-4-005198-0.
- [54] World Health Organization. *Laboratory Testing for the Monkeypox Virus: Interim Guidance*, 23 May 2022. Tech. rep. Geneva: World Health Organization, 2022. Chap. 6 p.
- [55] World Health Organization. Regional Office for South-East Asia. *Programme Budget Performance Assessment: 20202021*. Tech. rep. New Delhi: World Health Organization. Regional Office for South-East Asia, 2022.

- [56] *Definition of PREVALENCE*. <https://www.merriam-webster.com/dictionary/prevalence>. undefined.
- [57] *Diccionari Enciclopèdic de Medicina (DEMCAT). Versió de Treball / TERMCAT*. <https://www.termcat.cat/ca/diccionaris-en-linia/183>. -.
- [58] *Google Colaboratory - Alpha Fold 2*. <https://colab.research.google.com/github/sokrypton/-ColabFold/blob/main/AlphaFold2.ipynb#scrollTo=kOblAo-xetgx>. -.
- [59] *Immunology - YouTube*. <https://www.youtube.com/>. multiple.
- [60] *Interaction between Streptococcus Pneumoniae and Staphylococcus Aureus Generates ·OH Radicals That Rapidly Kill Staphylococcus Aureus Strains*. <https://journals.asm.org/doi/e-pub/10.1128/JB.00474-19>. -. DOI: [10.1128/JB.00474-19](https://doi.org/10.1128/JB.00474-19).
- [61] *Laboratory Notebook · Benchling*. <https://benchling.com/s/etr-sGhwNi3thI69pBb3Gw1g/edit?m=slm-1ZNe5iE4Txvx812cVgxw>. -.
- [62] *Microbiology/Infectious Diseases - YouTube*. <https://www.youtube.com/playlist?list=PLybg94GvOJ9HH55nc> -.
- [63] Center for Food Safety and Applied Nutrition. ‘BAM Chapter 12: Staphylococcus Aureus’. In: FDA (Wed, 05/13/2020 - 17:33).
- [64] *Promoting Biosecurity by Professionalizing Biosecurity*. https://www.science.org/doi/epdf/10.1126/science.aba0376?adobe_mc=MCMID%3D34422769753108397802497074084661275174%7CM-CORGID%3D242B6472541199F70A4C98A6%2540AdobeOrg%7CTS%3D1639589098. -. DOI: [10.1126/science.aba0376](https://doi.org/10.1126/science.aba0376).
- [65] *Staphylococcus Aureus Toxins | Elsevier Enhanced Reader*. <https://reader.elsevier.com/reader/sd/pii/S1369527413002191?token=A32ABB40B09CB72E7261B7B00541C8BF22151150B0A7472A8940E&ginRegion=eu-west-1&originCreation=20211215173133>. multiple. DOI: [10.1016/j.mib.2013.11.004](https://doi.org/10.1016/j.mib.2013.11.004).