

The Tale of a Bacteria Battle

*A study on Staphylococcus aureus, its prevalence, clinical possibilities and
our fighting tools*

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Final draft

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Contents

Acknowledgements	i
1 Prologue	3
2 Theoretical context	7
2.1 Bacteria and bacterial infections	7
2.2 The enemy: <i>Staphylococcus aureus</i>	8
2.3 The enemy's attacks	10
2.4 Our weapons	12
3 Physical experimentation	17
3.1 Description	17
3.2 Protocol followed	17
3.3 Bill of materials	18
3.4 Biosecurity and risk mitigation	19
3.5 Results and analysis	20
4 Computational experimentation	23
4.1 Description	23
4.2 Protocol followed	23
4.3 Results and analysis	24
5 Conclusions	25
5.1 Experimental conclusions	25
5.2 Bibliographic conclusions	25
5.3 Strengths and weaknesses	26
5.4 Possible improvements	26
References	I

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 - Protocol as published in protocols.io	XV
Annex 4 - Raw data table (data anonymised)	XXIII
Annex 5 - Bibliography consulted	XXV

1 Prologue

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

Stephen King

Our extremities are extremely important, and have been so since the dawn of time. They allow us not only to move about, but to socialise and tend to others, to express ourselves using art, to take care of ourselves, and many more things that we think are a given and thus, pay no attention to. That's why, when parts of them start to fail, humanity looked at its best friend, the brain, and asked it to develop a solution for this problem. And so it did: humans had created the artificial implant. A lot of injured people now could walk. However, a few months later, doctors realised that the zone around where they implanted the piece was hot to the touch and was starting to swell: typical symptoms of an infection. So, they treated it with broad-spectrum antibiotics, in hopes that this simple treatment fixed the ailment. And it did... that time. A few years later, after thousands of such infections, one patient stopped responding to antibiotics. And then another. And then a hundred more. The first antibiotic-resistant bacteria were born. So, after much studying, doctors, pharmaceutics and biologists developed a new compound to help with it. And it helped, until there were some cases in which it didn't. So the doctors, with the help of biotechnologists, turned to a fairly old line of research that had been dropped as soon as penicillin was discovered: the little bacteria-eating vira.

A few years later, summer of 2021, I had been accepted into a program at the Barcelona Autonomous University, which aimed to divulge microbiology and biotechnology to a group of 50 biology-loving students. That's where I learned in more detail about

bacteria, and how a microbiology/biotechnology lab functioned; and I fell in love with the discipline. I wondered how this magical-sounding research sounded, and thought it was a great fit for my Extended Essay.

This Extended Essay has as objective to study bibliographically the effects of *Staphylococcus aureus* on the human body, as well as the ways humanity has developed to defeat it. Experimentally, it has two main objectives, and several secondary ones: mainly, I want to find out the natural prevalence of *Staphylococcus Aureus* among my fellow schoolmates; as well as finding out the shape of the bacteriophage used to fight the most resistant strains of it, how it binds to bacteria and then reproduces using it; thus making the investigation questions "*What is the prevalence of Staphylococcus aureus in our school?*" and "*What proteins does the corresponding bacteriophage use to detect and bind to cells?*". Secondarily, this has the objectives of improving my lab etiquette and fluidity; allowing me to improve my protocols, how I follow them in the lab and how I deal with problems that may arise; making me discover how to work with extremely limited resources; practicing my tinction and microscope use; allowing me to practice analog translation of DNA into aminoacids; and last but not least, teaching me how to use AlphaFold.

This study requires taking samples from live human subjects. This is a one-off sampling process: they 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 documents the use of their data as well as an expected timeline for data anonymisation 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 data collection. The experimentation followed has no effect on the subjects.

¹See annex 1

²See annex 2

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)[1] as to find ways to mitigate any possible risk. During the experimentation there were 0 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 the used cotton swabs that were in the process of being disinfected. 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 bleach.

Before starting the experimentation, I had an interview with my coordinator in order to solidify the fact that there was no alternative to taking cutaneous samples from human beings, as well as a discussion on bacteria and the risks that this experiment implies.

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 animal kingdom. Their sizes range from between 30 μm and 100 μm 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[2].

Agar is used as a place to grow bacteria due to the fact that it is indigestible for the majority of bacteria, yet keeps them humid. Together with growth mediums, such as Lysogeny Broth, bacteria thrive in this environment, allowing them to proliferate and create colonies, which can be observed without the need of optic magnifying equipment. Sometimes, together with the growth medium, additives such as mannitol or salt are added. These are used to improve or impede bacterial growth, modify their conditions so they develop differently or as an identification tool. For example, *Staphylococcus Aureus* ferments the mannitol, producing acid, which in turn generates an acid and decolorates the plate's integrated pH indicator from red to yellow, whilst the salt prevents the growth of bacteria that are not of interest to the study[3].

¹Ubiquitous: found everywhere

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[2].

§2.2 The enemy: *Staphylococcus aureus*

Staphylococcus aureus (also known as Staph) is a GRAM-positive bacteria, the most studied and prevalent³ of its genus. Staph bacteria are usually harmless. However, they can 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 salting⁴) completely useless against said bacteria; a protein A capsid, which binds to many eukaryote organisms; as well as thermo-resistant enterotoxins. It's an extremely resistant (and thus ubiquitous) bacteria. It can be found in human skin and mucous surfaces (such as the mouth or the nose), as well as in certain foods such as ham (cooked or curated), eggs, raw and cooked dough, as well as in poultry.

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].

³"The degree to which something is prevalent: the percentage of a population that is affected with a particular disease at a given time"[5]

⁴citation needed

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. The plasma membrane consists of a lipid bilayer that is semipermeable⁵, 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 you know how to exploit it. Whilst the name implies PBPs are only sensible to penicillin, the name actually comes because that's how they were discovered, and in fact could be resistant to it but sensible to other 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. 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.

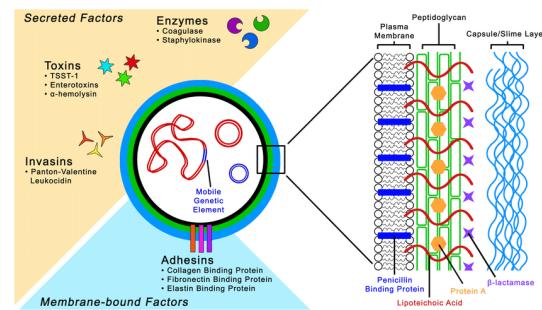


Figure 2.1: Parts of *Staphylococcus aureus*.
Source: PLoS Pathogens

⁵Semipermeable: it lets water and ions through, but not other molecules. This transport will always be in favour of the pressure gradient, which means that it cannot insert any kind of substance into an environment that has a higher pressure than the other side

§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. VISA and VRSA are what we would call a superbug, a microbe that has developed resistance to more quantity of antibiotic than is safe to consume. 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. This case is the bacteria equivalent of carcinisation, the discovery that several species have evolved into crabs; as well as the bacteria equivalent of tree leaves, which were developed independently by several species at the same time, in completely different parts of the world.

One of Staph's most notorious abilities is using the body's own proteins to disguise itself and thus avoid detection and phagocytosis by the host's immune system. It accomplishes this task by using enzymes called coagulases, which enable the transformation of fibrinogen⁶ to fibrin⁷[2]. Only 11 other *Staphylococcus* family members are coagulase-positive. To test for this enzyme in the laboratory there are two main methods which are usually combined: culture of the sample on a Baird-Parker agar medium, a selective and differential medium which contains lithium chloride and tellurite as to inhibit the growth of other microbes. It also includes pyruvate and glycine, which promote the growth of *Staphylococci* colonies, showing in colour black and with an opaque zone

⁶A glycoproteic complex produced in the liver and present in the blood of all vertebrates.

⁷fibrinogen after being stimulated by either thrombin or *staphylothrombin*, the result of a molecular pathway stimulated by coagulase. It helps in clotting the blood in the event of vascular or tissue injury

around the colony. This opaque zone represents the effect of the coagulase. Another way to test for coagulase is to perform a coagulase test. This test generally requires a small quantity (generally 2 mL) of sheep blood serum, which will gelatinise if coagulase is present.

Staphylococcus aureus contains an important quantity of **toxins**, which grants it 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. This category doesn't include, for example, those molecules intended to combat the host's defence mechanisms or scavenge reactive oxygen. We'll also exclude those situated on its membrane for the purpose of cell binding. Staph has several kinds of toxin in its arsenal: membrane-damaging toxins (which can be receptor-mediated or not), receptor-interfering toxins (not membrane-damaging), enzymes, and pathway blockers.

- Membrane-damaging toxins. Several of *Staphylococcus aureus*' toxins target the cytoplasmic membrane of the host's cells. These lead to pore formation in it, which provokes the outflux of vital molecules of the cell which, in turn, leads to cytosis⁸.
 - Receptor-mediated. Many of the cytolytic toxins of *Staphylococcus aureus* have been shown to require receptor interaction for their lytic activity. The best-known toxin of this kind is Alpha-toxin, also known as Alpha-hemolysin, which is its major cytotoxic agent, and is lytic to red blood cells and certain leukocytes, but not to neutrophils. Whilst at low concentrations it has been shown to be dependent on the interaction with cells' ADAM10 receptors, in higher concentrations of this toxin, this interaction is no longer necessary. Other toxins of this type include PVL (Panton-Valentine Leucocidin) and Gamma-toxin.
 - Non-receptor-mediated. In 2007, a toxin family that includes the Delta-toxin called the Phenol-Soluble Modulins (PSMs). PSMs trigger an inflammatory response by interacting with the FPR2 receptor, however they can carry cytolytic activity independently from FPR2 interaction. Delta-toxin has been linked to allergic

⁸Cell bursting due to osmotic pressure imbalance between the inside and the outside of it

skin disease and atopic dermatitis by degrading mast cells. This kind of toxin contributes to neutrophil lysis after phagocytosis, which might partly explain why the development of *Staphylococcus aureus* vaccines that work by enhancing a type of phagocytosis have failed so far.

- Receptor-function-interfering toxins. The toxins that fall into this category are enterotoxins⁹ These typically cause vomit and diarrhoea. *S. aureus* strains can produce a wide array (around 20) of entero and entero-like toxins. The most famous *Aureus* super-antigen¹⁰, the 22-kD toxic shock syndrome toxin (TSST), belongs to this group. TSS is a very severe and potentially fatal disease. *Staphylococcus aureus* also secretes a series of proteins that interfere with leukocyte receptors to evade recognition and thus activation of the immune system. CHIPS (Chemotaxis Inhibitory Protein of *Staphylococcus aureus*), which binds to the C5aR and FPR receptors, impairing the recognition of bacterial formylated peptides by the FPR receiver and blocking the activation of leukocytes via C5aR. *S. aureus* also has other proteins that work similarly to these, such as FLIPr.
- Enzymes. Many enzymes secreted by *Staphylococcus aureus* either degrade host molecules or interfere with its metabolic or signalling cascades. A few of them are proteases, which some non-specific ones have the ability to degrade host proteins in a broad proteins, leading to tissue destruction and necrosis, but may also have some more specific effects, for example the destruction of insulin B. Its two coagulases (staphylocoagulase and Willebrand factor) fall into this category.

§2.4 Our weapons

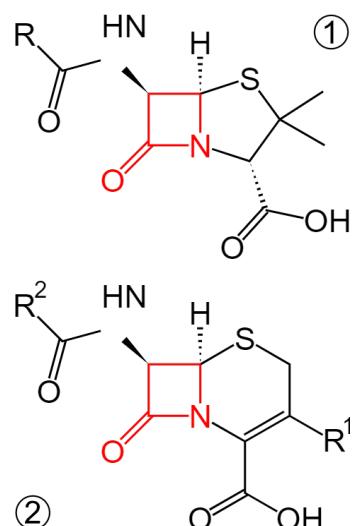
The tools we have at our disposal to fight off this infection fall into two main categories: chemical factors and biological factors.

⁹Enterotoxins are those toxins that target the intestines.

¹⁰Super-antigen: type of antigens that results in excessive activation by the immune system

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 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 latter, so they may be used before switching to a more specific 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 extremely 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.

It can be taken as a pill or as an injectable fluid, which proves it to be much more effective. This treatment is incompatible with aminoglycosides, a type of antibiotic that inhibit

Figure 2.2: Organic chemistry structure of penicillin (top) and cephalexin (bottom). -lactam ring in red.
Source: WikiMedia

protein synthesis, as it can lead to nephrotoxicity¹¹ and ototoxicity¹². vancomycin can induce platelet-reactive antibodies in the patient, leading to severe thrombocytopenia and bleeding with petechial hemorrhages on the tongue and bruises. Unfortunately, even with use of vancomycin, *Staphylococcus aureus* can develop resistance. In this case, no other option than using a biological factor is left. There has been one study in 2020 that discovered that by modifying the bacteria with a cationic oligopeptide¹³, vancomycin resistance could be bypassed. This could be a good solution temporarily as we wait for phage therapy to get approved, if it was a sufficiently studied option, which is not. This was discovered in 2020, while bacteriophage trials have been ongoing since the mid 2000s.



Figure 2.3: Organic chemistry structure of vancomycin. Source: Wikimedia

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 can lead to less side effects than antibiotics, as it only attacks a specific bacteria. This means, unfortunately, 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 class of virii has been studied since the late 19th century after being discovered by accident in

water from a river in India. This research, however, was dropped due to penicillin being discovered and put into effect, as the main purpose of this research was to use it on war casualties as to reduce mortality due to infections.

¹¹Nephrotoxicity: damage to the kidneys

¹²Ototoxicity: damage to hearing

¹³Cationic oligopeptide: sequence of two or more aminoacids that is positively charged

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 with 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 as 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.

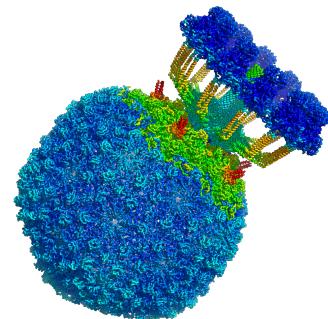


Figure 2.4: P68 structure.

Source: Own results

3 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

§3.1 Description

This experiment looks at finding out the prevalence of *Staphylococcus aureus* in a sample of students from the 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 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.

§3.2 Protocol followed

The protocol followed was designed based on a similar protocol used in the many university laboratories^[6], modified to fit the needs of this research paper, and peer-reviewed by Olga Sánchez, and uploaded to the Protocols.io platform, to make it easier to follow the days of that the experiment took place in. This protocol underwent 9 different revisions^[7]. It can also be read below:

- 1) Prepare yourself for the experimentation: wash your hands, put on gloves, put on the lab coat, mask and goggles. Wash your hands again (gloves still on). 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 wash your hands again.
- 2) Divide each Petri dish between 2 parts. A guide should be used for this part. Get your subject to wash their hands. Observe their hands. If they are extremely short, it may be worth it to take the sample nasally.
- 3) Note down their information, crack open a sterile swab, dip it in Ringer solution and swab away at under their nails or nose. Then, populate the dish with this sample.
- 4) Incubate for 32-48h and observe the results.
- 5) Observe the bacteria under a microscope after GRAM tinction.

§3.3 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, tinction 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		

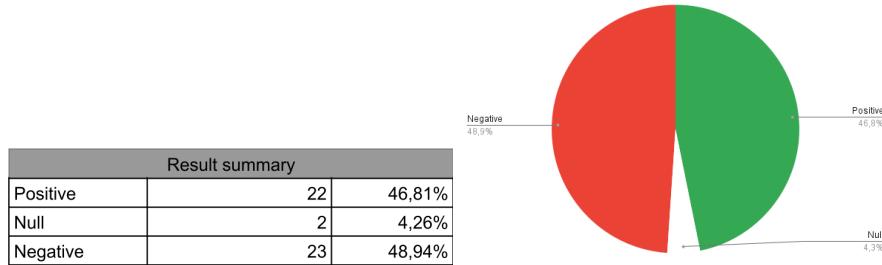
§3.4 Biosecurity and risk mitigation

S. Staph is considered a Biosecurity Level (BSL) 2 pathogenic bacteria[8]. This means that it is associated with a human disease that can pose a moderate human health hazard. In a laboratory where BSL-2 pathogens are handled, regular lab rules should be followed (mechanical pipetting only, hand washing, prohibition of the consumption of food and drinks in the lab, proper PPE use...), as well as avoiding splashes or aerosols, adhering biohazard warning signs present on all material used, as well as proper surface and material disinfection via the use of autoclave or proper alternative decontamination method[1].

The risks associated with this bacteria were assessed following the protocol designated by the World Health Organisation, and proper security measures were followed at all times when handling biohazardous material. No incidents occurred during the research part of this project, and the protocol defined previous to the start was followed extremely closely. While the laboratory used may not be the most ideal type of laboratory for this type of research, it was certainly adequate enough to perform a research project like this one, especially after the temporary signage that was temporarily installed.

§3.5 Results and analysis

The results obtained can be found as Annex II in the form of a raw data table with personal identificative data removed following GDPR regulations. The data was then recounted and graphed into the following pie chart:



(a) Counts of the result cases. Own data.
(b) Pie graph of the result cases. Own data.

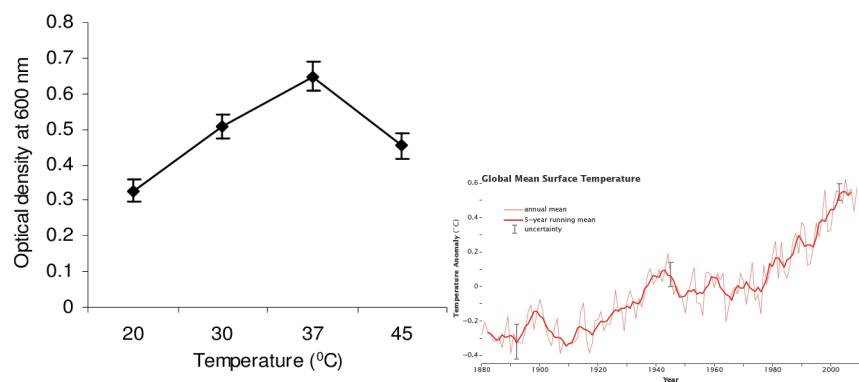
As we can see, almost 50% of the samples taken tested positive for *Staphylococcus aureus*, compared to the expected 30%[9]. We can, however, see in the UK's Public Health bacteraemia data that Staph infections have been on the rise lately, so it may not be a case of wrong data[10]. On top of that, both of my advisers, Olga and Margarita, have also found themselves getting more prevalence than usual of this bacteria, and are finding cases that were once negative but turned positive in the last few years.¹

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). They have seen a 210% increase in *Staphylococcus aureus* cases

¹insert table

since 2006. However, superfluous antibiotic prescriptions have increased by barely 1%[11]. In the United Kingdom, they have seen a 160% increase in *Staphylococcus aureus* infections[10], and their superfluous antibiotic prescriptions have gone down by 20%. While this seems like very little data to extract conclusions from, it is clear that there may be a correlation between these two factors.

The other could be climate change. An increase of ambient temperatures could mean a better breeding ground for this specific species and thus leading to a higher-than-usual prevalence. *Staphylococcus aureus*' optimal breeding temperature is between 35°C and 37°C. The global average temperature has increased by 1,1°C[12] in the last 120 years. And *Staphylococcus aureus* has a specific temperature growth curve, just like any other bacteria:



(a) *Staphylococcus aureus* growth curve by temperature[13] (b) Global warming plotted by the year[14]

Figure 3.2: Graphs relating the temperature of growth of *Staphylococcus aureus* and the increase of temperature of the Earth.

So, this correlation may not be completely incorrect, and in fact some scientists warn about an increased number of infectious diseases resurging due to climate change. One only data point is not enough significant data, so further study is needed on this front.

[bacteria observation] [this failed so I'm repeating it on Thursday at UdG]

4 Computational experimentation

Remember that all models are wrong;
the practical question is how wrong do
they have to be to not be useful.

Norman Richard Draper

§4.1 Description

This second experiment looks at computing the shape of a *Staphylococcus aureus* P-68 bacteriophage starting from its DNA sequence. This will analyse the process of protein translation, as well as looking at the process of using AlphaFold[15]. To compute the secondary, tertiary and quaternary structures of the proteic parts of the virus we will use the Google CoLaboratory version of AlphaFold[16], as it allows for far more computer power than available on a simple laptop.

§4.2 Protocol followed

- 1) Convert the DNA sequence of one of the proteins to an mRNA sequence.
- 2) Convert the mRNA sequence to an aminoacid sequence of one of the proteins.
- 3) Repeat for all the proteins that conform the virus.
- 4) Assemble the bacteriophage.

§4.3 Results and analysis

[Model was erroneous]

5 Conclusions

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

Michael Polanyi

§5.1 Experimental conclusions

This study has concluded that the prevalence of *Staphylococcus aureus* in our high school is 48,8%, 150% of 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.

It has also managed to produce a 3D printed figure of the bacteriophage that can help eradicate *Staphylococcus aureus*, regardless of antibiotic resistance, as well as discovering how the proteins bind to the surface of a bacterium, injects its DNA and then proceeds to its hosted reproduction.

§5.2 Bibliographic conclusions

While *Staphylococcus aureus* is a dangerous bacteria given the right conditions, most of the times, the immunitary 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 localised then that's when there is a problem. There are several strains of *Staphylococcus aureus*, classified by their resistance to antibiotics: MSSA (sensitive to meticillin), MRSA (resistant to meticillin), 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*

§5.3 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 realisation of the experimentation.

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.

§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.

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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

Principal Investigator: Pol Roca Cugat (ORCID: 0000-0001-8796-8396)

UDG Advisor: [TBD]

UAB Advisor: Olga Sanchez (NIU: 1004137)

Study Coordinator: Núria Feliu Pou, Departament de Llengües estrangeres.

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

Principal Investigator: Pol Roca Cugat (ORCID: 0000-0001-8796-8396)

Email: proca001@jvvgirona.eu

Study Coordinator: Núria Feliu Pou

Email: nfeliu001@jvvgirona.eu

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: Pol Roca Cugat (ORCID: 0000-0001-8796-8396)

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:

Pol Roca Cugat, 1Bat A Ins JVV. Email at proca001@jvvgirona.eu or for urgent contact send a private message via Discord at Peipr#8989.

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

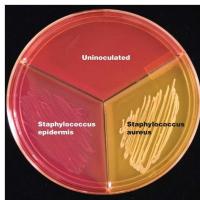
that this information was explained to you, your questions have been answered, and that you wish to continue participating in the study. If any new questions arise, you can contact the research team using the information provided above.

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 - Protocol as published in protocols.io



Staphylococcus Aureus Sampling V.10

Mar Roca Cugat¹, Olga Sánchez²

¹Institut Jaume Vicens Vives; ²Universitat Autònoma de Barcelona

Olga Sánchez: Peer review;

Version 10 ▾

Sep 19, 2022

dx.doi.org/10.17504/protocols.io.81wgb6pk1lpk/v10



Mar Roca Cugat

Institut Jaume Vicens Vives, Universitat de Girona

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ABSTRACT

This protocol is intended to study the affection of *Staphylococcus Aureus*, including the MRSA, VISA and VRSA variants, even if it makes the test more difficult to perform. It outlines the basic protocol for a multi-subject study, while using basic and minimal resources found in almost every biology lab.

DOI

dx.doi.org/10.17504/protocols.io.81wgb6pk1lpk/v10

PROTOCOL CITATION

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protocols.io
<https://protocols.io/view/staphylococcus-aureus-sampling-cguctwsw>
Version created by [Mar Roca Cugat](#)

KEYWORDS

Microbiology, sampling, swab, staphilococcus aureus

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CREATED

Jan 12, 2022

LAST MODIFIED

Sep 19, 2022

PROTOCOL INTEGER ID

70244

GUIDELINES

This protocol is intended to study the affection of *Staphylococcus Aureus*, which is a Biosecurity Level 2 bacterial agent. As such, the laboratory should be adequately to those standards or take measures in order to prevent infection, cross-contamination, or leaks.

MATERIALS TEXT

PPE

- Face shield or protective goggles
- FFP2/KN95 or higher-rated mask
- Latex, non-powdered gloves
- Lab coat

Sampling material

- Clean and sterile cotton swabs ($n+n/10$ being n the number of tests required)
- MSA Agar Petri dishes ($n/(2 \text{ to } 4)$ being n the number of dishes required)
- Sterile Ringer solution
- Permanent marker

Support material

- Bunsen burner
- Incubator
- Ethanol: >80%
- Bleach solution at 50%v in water.
- Reagents to make LB
- Inactivation buffer
- Photospectrometer

SAFETY WARNINGS

This protocol requires interaction with people, possibly infected with a pathogen (especially in 2022, when this protocol was written and put into practice, as COVID-19 was still going strong). As such, there is a risk of infection which can be minimized with proper PPE use.

Proper ventilation is recommended at all times, even when the pandemic situation is over. However, the sterile field must be preserved at all costs, so try to direct the airflow in order for it not to affect the results.

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Preparation

1h

10m



Wash your hands with soap. Put on your lab coat, your mask, and your goggles or face shield. Make sure your mask is airtight and air cannot escape through the sides.

- 1.1 Prepare the area where you are going to work. Disinfect the surfaces with the bleach solution.

The subjects should not be able to walk behind you, only to the side or to the front. Make sure to leave enough distance between the sampler and the subject, but not enough distance as for the sampling to be uncomfortable. The environment should be comfortable, within the following range of temperatures: **20 °C – 35 °C**

You should have a plastic, sealable box to your side or on the table to store the sampled Petri dishes.

The Bunsen burner should be to the front of you, within a hand of distance. The fresh swabs and Petri dishes should never be accessible by the subjects.

- 1.2 Using a permanent marker, divide each plate into two to four equal parts. You should help yourself by using a guide, such as a ruler.

- 2 Observe the subject's hands. If their nails are longer than 1-4 mm (the white part of the nail that can overgrow).
Bitten-down nails could lead to invalid results. Too long nails could lead to cross-contamination.
Ask the subject for their identificative and contact information if this has not been done previously.
- 3 Place a Petri dish on the side of the Bunsen burner. The burner will be the center of our sterile field.



Watch out so as not to break the sterile field

Step 3 includes a Step case.

Nail sampling

Nose sampling

step case

Nail sampling

For cases where the nails are 3-6mm long

- 4 Ask the subject to wash their hands thoroughly and below the nails with soap and lukewarm water. Note their subject ID on the bottom (agar side) of the Petri dish.
 - 4.1 Bring the subject's hands below the sterile area generated by the Bunsen burner. Open the Ringer solution and soak the swab. Proceed by swabbing below every nail in both hands. Once done, the subject can be dismissed.
 - 4.2 "Paint" one half of the Petri dish with the swab, softly so as not to break the agar but firmly as to get the sample to transfer to the plate.

5



1d

⌚ Repeat n times

Once the plate has 3 samples, place it in the full plates box.

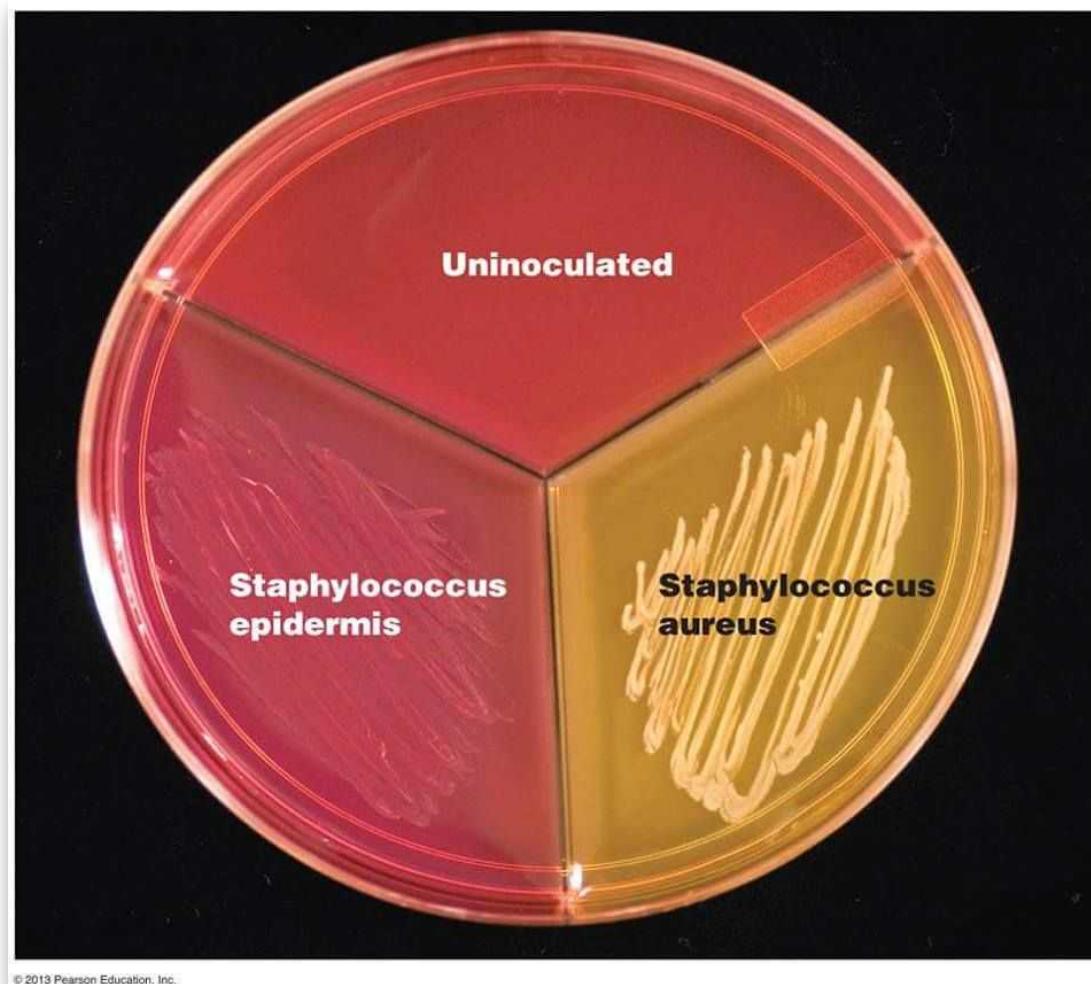
Once there are 20 plates in the box, group them together with tape, write an identificative group number and place it in the incubator.

The incubator should be set to **⌚ 37 °C** and left to incubate for **⌚ 24:00:00**.

Study

1d 2h

6



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It is expected to one of these results. A bright red colour means the sample was uninoculated. A pinkish colour with translucent streaks means there is *Staphylococcus epidermidis* present. A faint yellow colour or a bright yellow colour means there is *Staphylococcus aureus* present

The samples should be taken out of the incubator, the results introduced into the database and communicated to the subject.

7



Using one of the extra MSA Agar plates, culture and purify a sample of *Staphilococcus Aureus*. This can then be treated with GRAM tinture in order to observe it under an optical microscope (ideally at x1000-x1200).



Watch out for impurities/contaminations

Annex 4 - Raw data table (data anonimised)

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

Annex 5 - Bibliography consulted

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