The Tale of a Bacteria Battle

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

Mar Roca Cugat

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Ethical considerations (position TBD)

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 experiment[1] and a GDPR notice which documents the use of their data as well as an expected timeline for data anonymisation and destruction[2]. 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, and they were monitored during the process in order for them not to feel any kind of stress.

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) as 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.

[1] Can be found at https://biblio.peiphy.xyz/TDR-IC.pdf
[2] Can be found at https://biblio.peiphy.xyz/GDPR-notice.pdf

Abstract

Insert abstract here

Contents

1 Introduction

§1.1 An overview of the study

[I do not know what to write here. I don't even know if this should exist]

§1.2 Bacteria and bacterial infections

Bacteria are prokaryote organisms, generally single-celled, which are part of the Monera animal kingdom. Their sizes range from between 30 ţm and 100 ţm and are ubiquitous[1] 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 oxigen-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[MicrobioMed].
Agar is used as a place to grow bacteria due to the fact that it is indigestible for the majority of bacteria, yet it keeps them humid and, together with growth mediums, such as Lysogeny Broth, bacteria thrive in this environment, allowing them to proliferate and create colonies, which can be seen to the naked eye. Sometimes, together with the growth medium, additives such as mannitol 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, Staphyloccus Aureus ferments it, producing acid, which in turn decolorates the plate from red to yellow.

Pathogenic bacteria are bacteria that have the ability to cause disease[2]. These are not the most common type of bacteria, as the majority of them are either harmless or benefitial to the human body through symbiosis, such as the bacteria that help with digestion in the stomach[3].

[1] Ubiquitous: found everywhere
[2] "A 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."[dorlands:001]
[3] citation needed

§1.3 The enemy: Staphylococcus aureus

Staphylococcus aureus (also known as Staph) is a GRAM-positive bacteria, the most virulent and studied of its genus[4]. 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[5]) completely useless against said bacteria; a protein A capsid, which binds to many eukaryote organism. It's an extremely resistant (and thus ubiquitous) bacteria. It can be found in human skin and mucotic 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.

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.

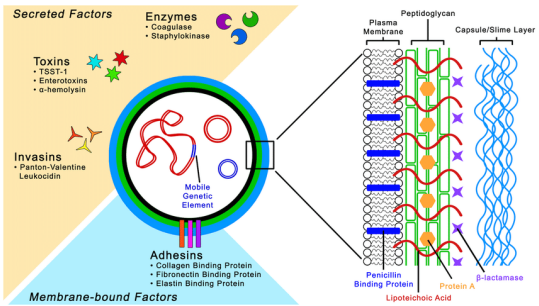

Figure 1.1: Parts of Staphylococcus aureus. Source: [source]

The plasma membrane consists of a lipid bilayer that is semipermeable[6], 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

[4] citation needed, got to check the proper terminology

[5] citation needed

[6] Semipermeable: 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

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.

§1.4 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 latterthe latter for which no antibiotic concoction that can erradicate 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 separetely, 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 coevolved 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 fibrinogen [7] to fibrin [8][source: microbiology medical]. Only 11 other Staphylococcus family members are coagulase-positive. To test for this

[7] A glycoproteic complex produced in the liver and present in the blood of all vertebrates.
[8] fibrinogen 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

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 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 cytolysis[9].
  - 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 inflamatory response by interacting with the FPR2 receptor, however they can carry

[9] Cell bursting due to osmotic pressure imbalance between the inside and the outside of it

cytolytic activity independently from FPR2 interaction. Delta-toxin has been linked to allergic skin sease 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 enterotoxins[10] 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 superantigen[11], 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.

§1.5 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 specific infection has before administering any antibiotic, as this treatment course will cause side effects such as killing gut bacteria, diminishing defense system capabilities, and increasing the possibility to develop yet more resistant infections. Generally, a large-spectrum antibiotic has an

[10] Enterotoxins are those toxins that target the intestines.
[11] Superantigen: type of antigens that results in excessive activation by the immune system

adequate risk level of causing the latter, so they may be used before changing to a more specific treatment. Starting with the least resistant general group, a -lactam antibiotic (such as methicillin, oxacillin, cloxacillin and penicillin) is the weapon of choice to fight against an MSSA infection. This is because the -lactam receptor in the cells mediate this contact, allowing the molecule to enter it and wreck havoc in the intruder. But once the -lactam ring is cleaved by an enzyme secreted by the bacteria itself, this type of antibiotic suddenly loses effect against them. That's where

§1.6 Risk assessment and prevention

Staph is considered a Biosecurity Level (BSL) 2 pathogenic bacteria. This means that the it is associated with a human disease that can pose a moderate health hazard. In a laboratory where BSL-2 pathogens are handled, regular lab rules should be followed (mechanical pipetting only, hand washing, prohibiting the consumption of food and drinks in the lab, proper PPE use...), as well as avoiding splashes or aerosols, biohazard warning signs present on all material used, as well as proper surface and material disinfection via the use of autoclave or alternative decontamination method.[source: book] The risks associated with this bacteria were assessed following the protocol designated by the World Health Organisation [cite], 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 to a T.

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Appendix