# Early sensing of bacteria – the fairytale version

Once upon a time, there was a deceptive bacterium that could hide in your body for several years and grow stronger and stronger before it could be discovered. Now, it is possible to diagnose it immediately when it enters your body.

And once upon a time, it could take up to five days from the point you delivered a sample until you received a diagnosis. Today, you can receive an answer in 30 seconds using a nanosensor. You may even be able to predict what the bacteria are intending to do in your body.

Does it sound like science fiction?

It is not. I managed to cover the whole process from idea development, sensor fabrication, technique development, laboratory validation and translation to a clinical trial on 62 patients – all within the 3 PhD years. The project resulted in a revolutionary method for the detection of one of the most problematic bacteria, way simpler, easier, faster and more sensitive than it is possible today. This method can allow more targeted and timely treatment of patients and potentially it can extend life expectancy and enhance life quality.

## Why this work can save the world

The threat of bacterial infections is rising to dangerously high levels. The United Nations declared antibiotic resistance to be one of the major threats to humans. Multi-drug resistant infections are estimated to cost health systems €53.2bn/year, i.e. 0.9% of global total health expenditure, killing more people than cancer in just three decades. Resistant bacteria will be the main cause of death and cost the global economy as much as €85.9 trillion by 2050. As a result, The World Health Organization (WHO) insists on urgent need for affordable, sensitive, specific, user-friendly and rapid diagnostic tools and alternative ways of infection management.

## Snitching on a snitch

The troublemaking bacteria I worked with is the so-called *Pseudomonas aeruginosa*. WHO has classified *P. aeruginosa* as one of three highly critical pathogens that pose the greatest threat to human health. It is opportunistic, meaning that it is sneaky enough to lay low if you are healthy, but if you are vulnerable, it will take advantage of your situation and make you seriously sick.

But how do bacteria decide to make you sick? Bacteria communicate with each other using signaling molecules that allow them to synchronize their behavior. If bacteria single-handed try to release molecules to make you sick, it will have no effect on your huge body. Only, when bacteria are many enough, their behavior can make a difference to you. This system is called quorum sensing and allows bacteria to secrete molecules that other bacteria can sense and respond to by specific actions. E.g. bacteria could decide to attack the human body by releasing toxic molecules, or attacking competing bacteria by physical violence. These signaling molecules are often not recognized by the immune system, why the human body is often taken by surprise when an infection breaks.

One of the molecules bacteria release when they decide to make you sick is pyocyanin. Pyocyanin is a toxin, that can only be produced by *P. aeruginosa*. It is a snitch telling that *P. aeruginosa* is present. The job of the nanosensor I developed was to snitch on this molecule. If the nanosensor could sense pyocyanin in a patient sample, the sample was positive for *P. aeruginosa*.

## Patients thought they were cleared

I tested my nanosensor in a clinical trial that included 62 patients with the rare disease cystic fibrosis. Patients with cystic fibrosis have a genetic disorder that makes their lungs work as a trap for bacteria. *P. aeruginosa* is responsible for the death of 80 % of the patients that only have an average life expectancy of 37 years. The premise was that if there was pyocyanin in their sputum samples, then the bacteria must exist in the lungs of the given patient. I compared the sensor results to the usual diagnostics made by the laboratory of the hospital and conducted DNA identification of this bacteria, which is a difficult but conclusive evidence for the existence or no existence of the bacteria.

What I found was astonishing.

61 % of the patients had an infection that was never caught by routine analysis. The patients literally went home thinking they were free from infection while the bacteria were building up in their body. Many of these patients became positive in later routine visits. The sensor could foresee that a positive diagnosis was upcoming.

But there is more to this story, because along the way other important findings were done. Once the nanosensor was used on patient samples, a whole other scene appeared. I ended up proving that in the majority of the cases, patients who were clinically described as free from bacteria after antibiotic treatment actually were still infected.

The yearly number of patients with first-time *P. aeruginosa* infections among patients with cystic fibrosis is usually reported to be 1-2 %. However, I found 10 % first-time infections in the clinical trial. Thus, the nanosensor could give us information that was impossible to obtain with the current methods. The nanosensor embraced an on-the-spot technique for early diagnosis of *P. aeruginosa* directly in patient samples with immediate answer and with the precision of DNA analysis.

## Caught with the hand in the cookie jar

The sensor can catch the bacteria, but can it also tell us what the bacteria are doing? Are the bacteria about to make the patient very sick or are the bacteria just harmless? This is something the sensor can reveal by quantifying the toxins the bacteria secrete.

Sensing bacterial conversations is not only fascinating from a clinical point of few. From a fundamental microbiology perspective, it is of outmost interest to follow bacterial signaling to each other as it happens. I designed and fabricated a device where I could grow bacteria and measure online and in real-time how the bacteria reacted to different stresses and loads. Following bacterial activities live may bring a whole new dimension to the understanding of microorganisms that is not possible with the methodologies available today.

## And now what?

After three years of research, I had developed and validated a nanosensing method that was capable of identifying a specific problematic bacterium.

So far so good. But where do we go from here?

The nanosensing I have developed may be expanded to other pathogens that are important to diagnose immediately. Bacteria are susceptible during the early infection stages as they have not grown strong yet. The earlier we diagnose dangerous bacteria, the more success we will have treating with antibiotics. If we eradicate the bacteria early, we may avoid the development into chronic infections and this will significantly reduce the antibiotic use in the long run.

Nanosensing can be used to confirm if a treatment has completely eradicated bacteria. Close monitoring of the infection status of patients with nanosensing will allow prescribing personalized treatment. As the nanosensor can determine both the presence of the bacteria and what the bacteria are doing, it may be possible to make individual treatment strategies depending on how the patient condition develops.

Now, the vision is to expand the nanosensing to other microorganisms. Not only pathogens that are dangerous to the human health but also to the healthy bacteria we are co-existing with. The composition of bacteria within us is believed to influence our health, our weight and even our mood. We should aim at understanding the bacterial signaling molecules in order to sustain a healthy bacterial composition in our bodies.

Using nanosensors that can monitor the behavior of our microorganisms, simply from spit, blood or urine samples at home, we may be able to control our own bacteria and direct their actions in our favor. And ultimately it may help us live happily ever after.

# References


1. Marvig, R. L., Sommer, L. M., Molin, S. & Johansen, H. K. Convergent evolution and adaptation of Pseudomonas aeruginosa within patients with cystic fibrosis. *Nat. Genet.* **47,** 57–64 (2014).
2. Jayaseelan, S. & Ramaswamy, D. Pyocyanin: production, applications, challenges and new insights. 1159–1168 (2014). doi:10.1007/s11274-013-1552-5
3. Lau, G. W., Hassett, D. J., Ran, H. & Kong, F. The role of pyocyanin in Pseudomonas aeruginosa infection. *Trends Mol. Med.* **10,** 599–606 (2004).
4. Reyes, E. A. P., Bale, M. J., Cannon, W. H. & Matsen, J. M. Identification of Pseudomonas aeruginosa by pyocyanin production on Tech agar. *J. Clin. Microbiol.* **13,** 456–458 (1981).
5. Alatraktchi, Fatima AlZahra'a et al. "Paper-Based Sensors for Rapid Detection of Virulence Factor Produced by Pseudomonas Aeruginosa." Ed. Yogendra Kumar Mishra. P L O S One 13.3 (2018).
6. Al Atraktchi, F. A.-Z., Johansen, H. K., Molin, S., & Svendsen, W. E. (2016). Electrochemical sensing of biomarker for diagnostics of bacteria-specific infections. Nanomedicine, 11(16), 2185–2195.