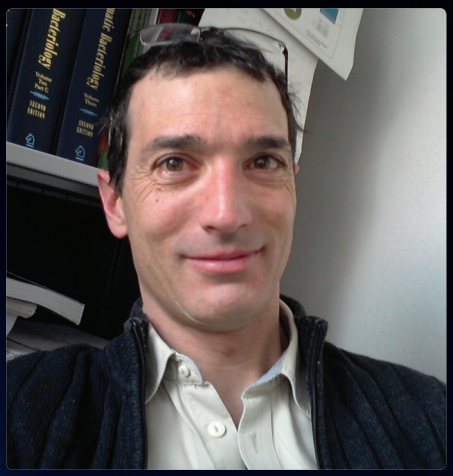
Contaminated **treatments**

DR KARL PERRON

Metals commonly used in medical devices and food supplements may render virulent infections resistant to antibiotics. **Dr Karl Perron**'s laboratory is examining the mechanisms underlying this resistance



Could you put into context the implications for human health of bacterial resistance to antibiotics caused by toxic metals?

About 15 million people die each year from infectious diseases, many of which were once treatable with antibiotics. The emergence of antibiotic-resistant bacteria is a crisis for public health. There is an urgent need for new antimicrobial drugs and to understand the mechanisms that lead to the selection of antibiotic resistant bacteria.

Zinc and other metals are often used in medical devices, such as catheters, and in pomades, creams and nutritional supplements. Our research reveals that the presence of these metals may not be harmless and caution is needed: metals may activate antibiotic resistance in bacteria, which might explain some therapeutic failures.

Why have you chosen to study the bacterium *Pseudomonas aeruginosa*?

P. aeruginosa is naturally present in our environment and is frequently found in soil and water. It causes about 30 per cent of nosocomial infections, severely affecting burns victims, cystic fibrosis and intubated patients as well as immuno-compromised people, and is naturally strongly resistant to numerous antibiotics.

We observed that it could resist high zinc concentrations and started to study its resistance mechanisms. Because it is also a severe pathogen, we then studied its antibiotic resistance profile and found that zinc induced its carbapenem antibiotic resistance.

What are two-component systems? How do they contribute to antibiotic resistance?

Two-component systems are the major mechanisms by which all bacteria detect and respond to changing environmental conditions and stresses. They act like a switch that, upon sensing external or internal stimuli, induces a phosphorylation cascade, enabling the bacterial cells to modulate gene expression and adapt their physiology in a specific and rapid manner. Some contribute to pathogenicity and others to antibiotic resistance.

P. aeruginosa possesses about 130 genes encoding for two-component systems, a huge number, that allows it to adapt to many diverse environments, thereby contributing to its versatility.

What is your pivotal finding to date?

Our discovery of a new co-regulation mechanism between metals, antibiotics and virulence opens up major new areas of research into *P. aeruginosa* and probably many other pathogens. It is a key discovery, and is currently the major topic for my laboratory.

What inspired you to set up the Microbiological Analytical Platform, a core facility in microbiology?

More and more researchers needed competence in bacteriology for diverse experiments on class two pathogens. We perform microbiological or sterility controls following structured procedures and screen for new antibacterial compounds. We offer expertise and the opportunity to conduct research safely, in a controlled manner. Due to increased demand, we are currently seeking funding to expand our services.

Could you expand on your collaborations with pharmacologists to translate your work into treatments for common pathogens?

We started screening libraries of compounds isolated from plants used in traditional medicine to test their putative antibacterial properties. We focused first *Staphylococcus aureus* and *P. aeruginosa.* For both, there is a need for new therapeutic drugs. We identified several promising anti-Gram positive compounds in our platform.

We are currently designing another screening procedure, not to kill *P. aeruginosa*, but to render it harmless. Quorum-sensing is important for its pathogenesis, since mutants without this communication system show decreased virulence. So, using molecular techniques, we are building a screen to discover compounds able to block *P. aeruginosa*'s quorum sensing communication and render it non-virulent. This type of research is crucial for finding new anti-infection molecules.

As manager of BiOutils, do you believe that widening the accessibility of the sciences is important for society?

The main goal of BiOutils is to provide support to teachers so that they can perform modern biology experiments in their classrooms. We also organise conferences and events. It is important to promote the sciences but more so to provide information about new discoveries: progress is so rapid and the implications for humankind are so important that they need to be explained and taught to all, young and old.

How will you focus your investigations over the next six months?

Since we know that in certain conditions, for example in the sputum of cystic fibrosis patients, excess metals might be found, we will investigate whether this conduces to infection.

We have also observed that the control of the OprD channel, involved in the entry of carbapenem, is controlled by metal dependent mechanism involving small regulatory ribonucleic acid, and we are focusing on its workings.

DR KARL PERRON

Pathways to infection

A study is currently underway at the **University of Geneva** into the role of some heavy metals in rendering opportunistic pathogens insusceptible to certain antibiotics. It presents a nightmare scenario of unbridled superbug infections that resist modern medical treatments and progress unimpeded across populations

SINCE BACTERIA ARE not only all around us but also within us, it is inevitable that some will proliferate to the detriment of our health when our natural resistance is low due to immunological deficiency or vulnerability from injury or disease.

Cystic fibrosis sufferers and people who have sustained severe burns are highly vulnerable to infections from the opportunistic pathogen, *Pseudomonas aeruginosa*. This bacterium can survive in conditions depleted of oxygen, such as those in infections in cystic fibrosis sufferers' lungs; *P. aeruginosa* is also intrinsically resistant to antibiotics and the last line of treatment for advanced infection by it and other bacterial infections such as *Escherichia coli* is often the antibiotic carbapenem.

While outbreaks of superbugs like *E. coli* in hospitals in recent years have been linked to a need for greater cleanliness and the wider use of antibacterial cleaning products in an attempt to inhibit cross-contamination, Dr Karl Perron of the Microbiology Unit at the University of Geneva considers that there may be a more insidious contributor to the resistance of some bacteria to carbapenem and thus a key contributor to the incidence of nosocomial infections in hospitals: the use of metals in some medical equipment and treatments. Perron and his team have discovered that bacterial cell behaviour is altered by the presence of any of a number of trace metals such as zinc, cobalt and copper; and the equipment used in hospitals for treating diseases and infections often includes elements made from metals such as these.

THE BORDEAUX MIXTURE

Copper has been used for many years in agriculture as an anti-fungal treatment. As a result, much agricultural land contains elevated levels of copper: "The Bordeaux Mixture, comprising copper sulphate and calcium oxide, for example, has been dispersed in vineyards for more than a century to fight mildew," explains Perron. While metals contribute to the function of some enzymes, they are ordinarily toxic for all living organisms in high density. However, environmental microorganisms provide the first line of defence against toxicity in the environment and bacteria have evolved mechanisms to enable them to thrive in even highly contaminated conditions.

Perron's research into the roles of metals in conferring resistance to antibiotics on bacteria started several years ago during a prospective study of bacterial flora in soil in the Geneva area. Finding that the pathogen *P. aeruginosa* was present, Perron and his fellow researchers started to investigate its mechanisms for resisting metal toxicity: they found that the bacterium's efflux pump expels metals, and this allows it to thrive in soil contaminated with metals.

They discovered that *P. aeruginosa* detects metals via a two-component system, the type of signalling system that bacteria use to adapt to changes in their environment. Two-component systems have a sensor that, on receipt of a signal, transmits information to a regulator that then prompts an adaptive response. *P. aeruginosa* has many of these systems and Perron concluded that this explained its adaptability. The group also found that one of the two-component systems, known as CzcRS, can detect zinc, cadmium or cobalt and then 'switch on' the efflux pump to expel the metal from the cells. To their surprise, they then observed that when the sensor system was activated by metals, the bacterium

Such huge complexity in so small a cell is fascinating

also became resistant to carbapenem; it was from this that they deduced that a co-regulation mechanism governed both metal and antibiotic resistance in *P. aeruginosa*.

The CzcRS system regulator, when activated by metals, was able to repress the expression of a membrane protein, a porin named OprD, which otherwise provides the channel of entry for carbapenem into bacterial cells. Therefore, in the presence of metals, the bacterium became completely resistant to carbapenem. More importantly, they observed that the exposure of *P. aeruginosa* to high zinc concentrations led to resistance to both zinc and carbapenem, even after the zinc was removed, which suggested to them that metals can in fact render *P. aeruginosa* permanently resistant to carbapenem.

CURRENT FOCUS

These findings were of particular concern since the clinical strains of *P. aeruginosa* under test were no different from their environmental counterparts. The presence of zinc in contaminated soil or rivers could render the bacterium resistant to carbapenem even without exposure to the antibiotic. Perron's laboratory then tested latex urinary catheters, which contain zinc, and found that zinc from them was released into urine in sufficient amounts to induce carbapenem resistance in *P. aeruginosa*. The conclusion was reached that use of those catheters could compromise carbapenem treatment of urinary tract infections by *P. aeruginosa*. In recognition of the environmental and medical implications of this work, Perron was awarded the 2005 Pfizer Prize for Infectiology. Work was then focused on the effect of copper on *P. aeruginosa*, with equivalent results. In view of the widespread application of copper in agriculture and viticulture, Perron now plans a prospective study to investigate antibiotic resistance in *P. aeruginosa* isolated from soil treated with copper.

The investigations also recently revealed that the two-component system involved in the responses of *P. aeruginosa* to metal also modulates its pathogenicity: if the CzcRS switch is disrupted at the molecular level, the bacterium's virulence decreases substantially. The CzcRS switch seems therefore to be directly involved in the control of several key genes that govern quorum sensing and virulence activity of *P. aeruginosa*, and the switching controls are now the main focus of research: "To understand the molecular mechanisms of CzcRS that govern metal tolerance, carbapenem resistance and modulate the virulence of *P. aeruginosa*, we use genomic techniques such chromosome immunoprecipitation followed by high throughput sequencing. And we have a sophisticated proteomics analysis underway to obtain a map of all proteins regulated by the CzcRS two-component system," Perron elucidates.

UNRAVELLING COMPLEXITY

Perron's discovery of co-regulation mechanisms in bacteria that coordinate different responses indicates that many unanswered questions remain. "We are working on a very common and well studied bacterium, but the *P. aeruginosa* example is a very intriguing case. We don't know yet why these resistances are interconnected," he reflects. "Our results suggest that two-component systems are not specific to one challenge but integrate different cellular responses. More importantly, our work shows that metals might enhance not only antibiotic resistance but also modify the virulence of certain bacteria."

It is hoped that this research will firmly establish that metals are risk factors in infectious diseases and at the same time expand understanding of the complexity of the regulation mechanisms of bacteria, but much more analysis is needed. There are estimated to be many billions of billions (10^{30}) of bacteria, yet fewer than 1 per cent are known. The question of how and why specific responses are linked to others seems to be a simple question on the surface, but in fact the answer is much more complicated, as Perron expands: "With only five or six thousand genes, we thought that we could easily understand bacteria. However, many pathways are interconnected, allowing bacteria to adapt and respond to many diverse challenges. Such huge complexity in so small a cell is fascinating".

DR KARL PERRON, DR GUENNAËLLE DIEPPOIS, SANDRINE ZUCHUAT, TIZIANA SCRIGNARI, VÉRÉNA DUCRET

INTELLIGENCE

INDUCTION OF ANTIBIOTIC RESISTANCE IN PSEUDOMONAS AERUGINOSA BY TRACE-METALS POLLUTANTS

OBJECTIVES

The main objective is to understand how metals, present in contaminated area or in medical devices, might increase antibiotic resistance of bacterial pathogens. Work to decipher the complex regulatory pathways that link metal resistance to antibiotic resistance and virulence using molecular methods will be conducted.

KEY COLLABORATORS

Team members: **Professor Patrick Linder**, CMU, University of Geneva, Switzerland • **Professor Karine Lapouge**, DMF, University of Lausanne, Switzerland • **Dr Mélanie Marguerettaz**, **Véréna Ducret**, **Sandrine Zuchuat**, **Tiziana Scrignari** and **Carolyn Heckenmeyer** • Former postdoc student: **Dr Guennaëlle Dieppois** • This work was initiated in collaboration with **Dr Claude Rossier** and **Dr Olivier Caille** and was pursued in part in the lab of **Professor Christian van Delden**

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KARL PERRON is group leader in bacteriology and drives the research in the field of antibiotic and metal resistance in *Pseudomonas aeruginosa*. He is also in charge of teaching theoretical and practical courses in bacteriology. In addition he manages the BiOutils interface for the development of secondary school-university interactions and to promote life sciences. Perron collaborates within the centre of excellence of bacteriology (CEBUG) to stimulate research and interaction on bacteriology in the University of Geneva.

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