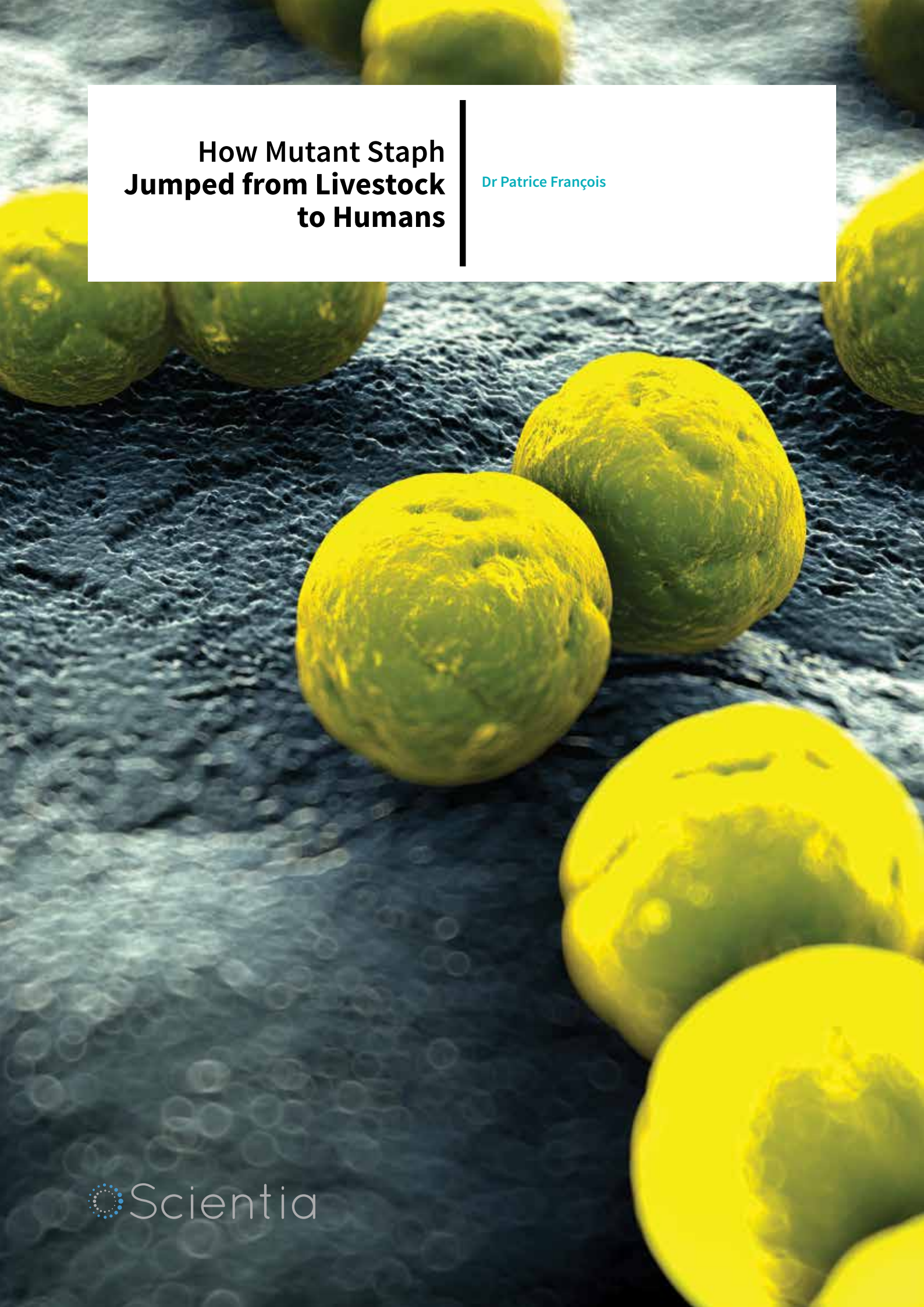
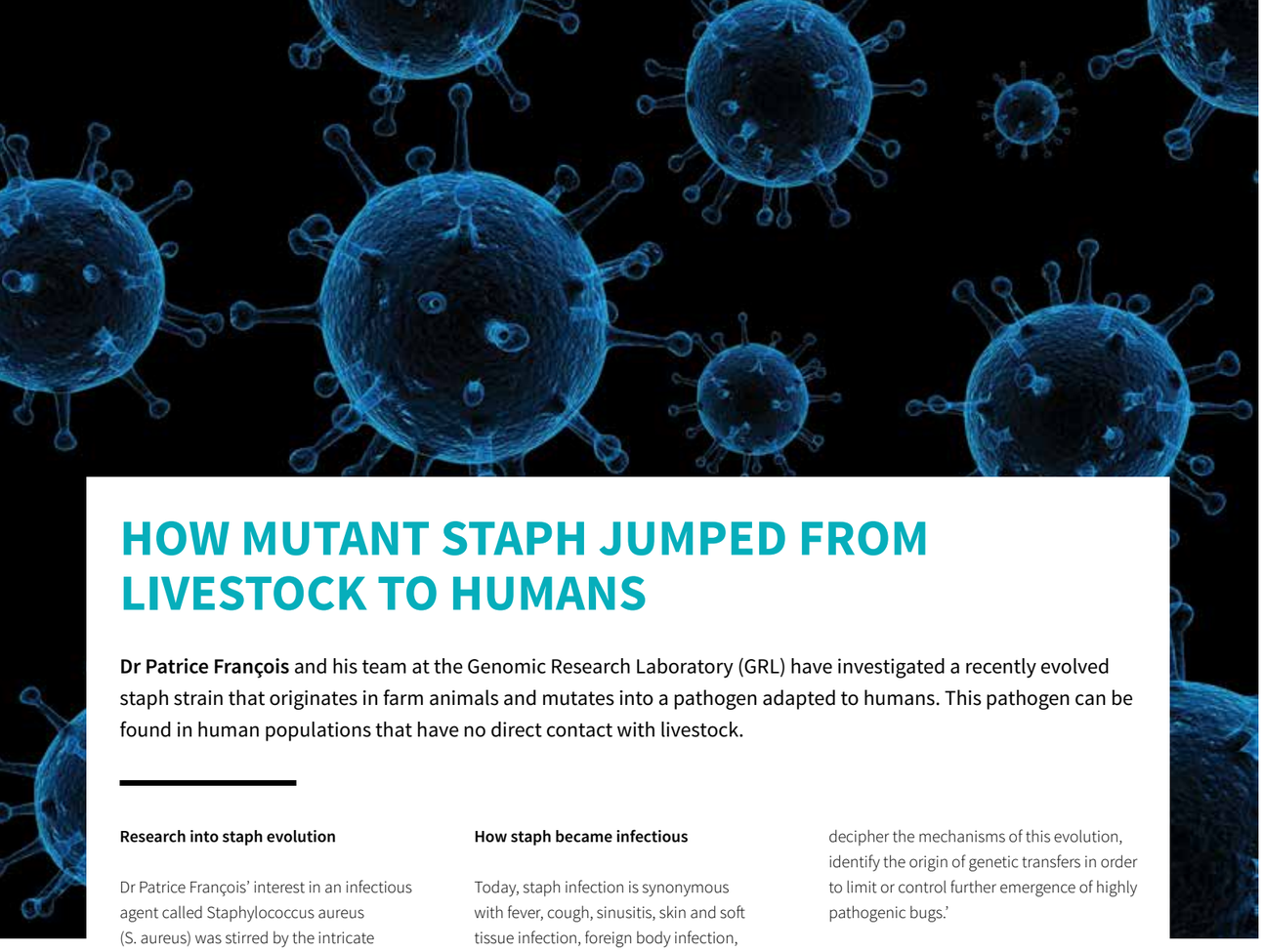


How Mutant Staph Jumped from Livestock to Humans

Dr Patrice François

Scientia



HOW MUTANT STAPH JUMPED FROM LIVESTOCK TO HUMANS

Dr Patrice François and his team at the Genomic Research Laboratory (GRL) have investigated a recently evolved staph strain that originates in farm animals and mutates into a pathogen adapted to humans. This pathogen can be found in human populations that have no direct contact with livestock.

Research into staph evolution

Dr Patrice François' interest in an infectious agent called Staphylococcus aureus (S. aureus) was stirred by the intricate circumstances surrounding the evolution of bacteria, and the medical potential of such discoveries. Describing the significance and scope of his research, François tells Scientia: 'Since 2000, we are using strictly the same protocol of survey for patients suffering bloodstream infections due to S. aureus. We noticed an evolution with the emergence of a specific clone of S. aureus responsible for severe infection in humans. Initially, this clone was restricted to pigs or pig farmers and was a poorly pathogen, meaning that it was mainly found in asymptomatic carriers. Suddenly, variants of this clone were found in severe infections in humans. The starting point of this evolution is, probably, industrial livestock. This evolution appears associated with the acquisition of mobile genetic elements by "naïve non-pathogenic strains" contributing to modify host tropism and to an increased pathogenicity in humans.' To test their working hypothesis, François and his team infected bacteria with viruses and followed its behaviour to see whether phenotypic changes occur.

How staph became infectious

Today, staph infection is synonymous with fever, cough, sinusitis, skin and soft tissue infection, foreign body infection, osteomyelitis or endocarditis. However, this has not always been the case. In fact, staph bacteria began as being a harmless colonizer and grew, by insertion of viral genetic material, into the super-resistant, dangerous pathogen that it is today. In this context, Dr François' work carries a lot of weight because it can offer new insights into the mechanisms of how bacteria mutate to become pathogenic, and how resistance to antibiotics arises. Moreover, such pathogens possess a high potential to resist treatment due to the presence of many different, fast mutating strains.

Following mutation, pathogenic bacteria that arise can further mutate, evolving into increasingly dangerous pathogens that cause severe illnesses. The path towards gaining a better understanding of the process resulting in new bacterial behaviour is twofold. Researchers are investigating the way by which bacteria acquire new genetic elements from viruses in their attempts to discover the exact evolutionary mechanisms that give bacteria their harmful characteristics. In Dr François' words, the aim of this research is 'to decipher the mechanisms of this evolution, identify the origin of genetic transfers in order to limit or control further emergence of highly pathogenic bugs.'

What makes the staph infections dangerous and, implicitly, opens up highly productive avenues of research is the symbiotic combination of viruses and bacteria. Viruses cannot reproduce by themselves, and do so by depositing genetic instructions into living cells, meaning that they are not technically life forms. However, viral genetic material present in infected bacteria can turn them into new strains transmittable with increasing ease at each new generation. Further to that, their characteristics become more harmful for human hosts.

Acquiring mobile genetic elements

Within the last twenty years, a strain that first colonised, and later infected, pigs and livestock, called S. aureus clonal complex (CC) 398, has evolved the ability to colonise and infect humans. S. aureus CC398 was already a worldwide problem for the farming industry. Now, since it has evolved and crossed over to humans, even people who have not had direct contact with livestock or farms have been found to have contracted the disease.

For a better understanding of the inner workings of the staph evolution by the insertion of genetic elements, a few observations are in order. A genome is the complete set of genetic material made up of DNA inside the nucleus of a cell, and is the target of this study, since it carries all the genetic information necessary for replication and evolution. Dr François studied how this subpopulation of CC398 bacteria has evolved to infect humans by acquiring mobile genetic elements from viruses. Bacteriophage viruses – or phage in short – are types of viruses that freely infect bacteria and use them to reproduce. Pieces of viral genome inserted and, later on, integrated into the genomes of the next generations of bacteria are called prophage. Interestingly, at the stage where the prophage genome is integrated into the bacterial genome, it is no longer harmful or disruptive to the functioning of the host.

In the case of CC398, a genetic element was originally inserted by a virus into a bacterial cell and is now present inside the cell alongside the original CC398 genome. Since the prophage is actually the genetic material of the virus itself, it causes changes in the observable characteristics of the bacteria. A prophage can be integrated directly into the main bacterial genome, as is the case for CC398, and this type of phage is known as a 'lysogenic phage'. Prophages are a very important element of bacterial diversity and evolution. They are able to act as an evolutionary driving force for bacteria, allowing the development of new strains that are adapted to new environments.

Understanding pathogenic behaviour

S. aureus is a bacterium that causes acute and chronic infections in humans and animals – in particular, infections of the skin and respiratory tract. Presently, there are many known strains of S. aureus, some of which do not respond well to classic antibiotics. In their recent work, Dr François and his team have used novel tools in genetics research, such as high-resolution whole-genome microarrays, prophage profiling, immune evasion cluster characterisation and whole-genome sequencing to investigate prophages in human-adapted CC398. With these tools they were able to study a bacteriophage virus (or phage) and two different prophages that are harboured by human-adapted CC398.

Under the influence of new mobile genetic elements, or from the interaction of the

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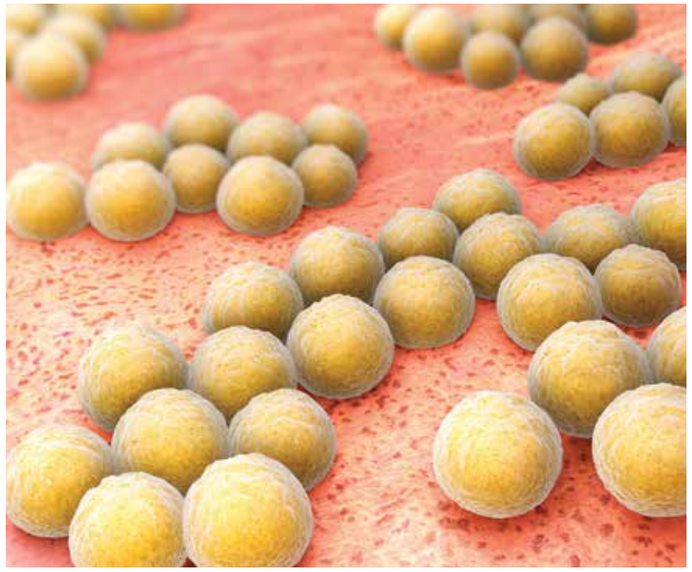
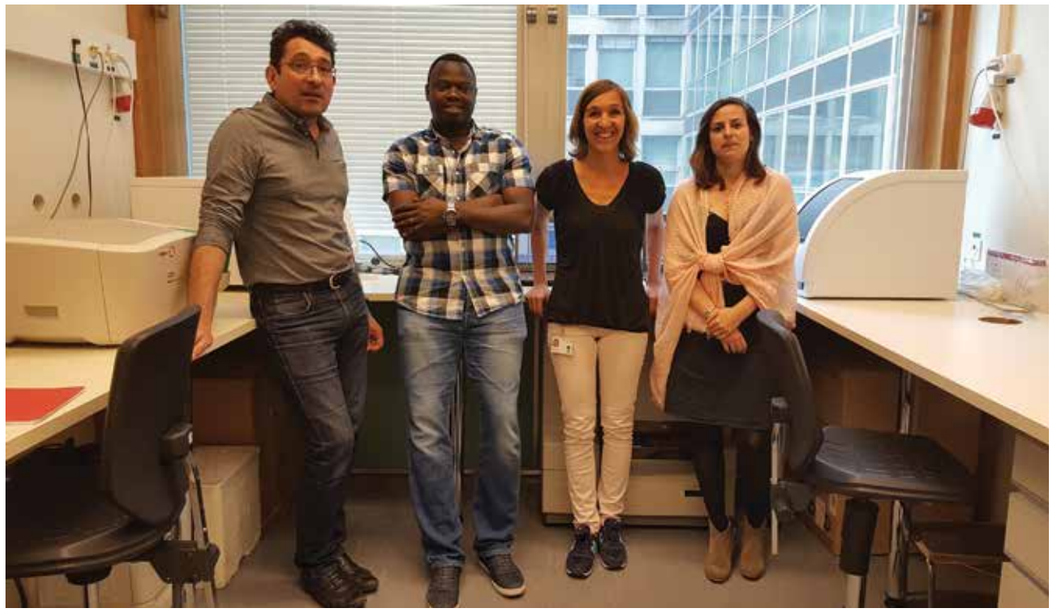


genotype with its surroundings, bacteria develop new observable characteristics and behaviours as part of the evolutionary process. While the genotype of an organism refers to its genetic constitution, the phenotype is synonymous with the set of new behaviours and interactions observed after infection. In order to study the process of phenotypic modifications by genotypic augmentation, the researchers inserted phages into isolates of CC398 that originally did not contain prophages, or in other words, isolates not yet adapted for becoming infectious in humans. One of the interesting phenomena they investigated was the range of effects that the phage's reproductive cycles have on the CC398 host. This was performed by studying a process called lysogeny, a phage reproductive cycle in which viral nucleic acid becomes embedded in the bacterial genome. By following the lysogenic cycle, researchers were able to investigate the resistance of CC398 to further phage infection, how CC398 evolves the ability to colonise human cells, and the virulence factors that are expressed by the phage genome once it is integrated.

Virulence factors are molecular by-products that arise due to instructions that are encoded in the phage genome which hijack the cellular machinery of the host cell. These molecules create favourable environmental conditions for the phage and, in turn, induce many of the symptoms experienced by the organism carrying the viral disease – in this case humans. In their research, Dr François' team infected staph with viruses, starting from the working hypothesis that bacteria became pathogenic after being infected by viral genetic material. The purpose of the experiments was to see how infected bacteria behave after infection, how the genome becomes fixed into the new form, and to



The team. from left to right: Patrice Francois, Seydina Diene, Eve-Julie Bonetti, Floriane Laumay.

study virulence factors induced by bacteriophage viruses into bacteria.

In the work, a total of 21 CC398 isolates were studied in two groups of livestock associated (LA) and non-LA bacteria. Dr François and his team found that in non-LA CC398, a prophage called StauST398-5pro offered significant protection of the host bacteria from horizontal genetic transfer to the host. Horizontal genetic transfer is a process where bacteria transfer genetic material directly to each other, which strongly contrasts with the normal parent-offspring means of genetic transfer. Horizontal genetic transfer includes the phage to prophage process that resulted in the evolution of the human-adapted CC398. The prophage StauST398-5pro was also found to influence virulence genes so that they are expressed in stress situations.

In 1961, an antibiotic-resistant strain of S. aureus was discovered, now known as methicilin-resistant S. aureus shortly after the introduction of the drug in human medicine. More prominently known by the acronym MRSA, today the resistant staph strain has become a global problem. MRSA evolved from a methicilin-sensitive predecessor that was not resistant to methicilin antibiotics, called MSSA. Of the 21 CC398 isolates studied, the majority of strains were MSSA, as opposed to MRSA.

Future research directions

Dr François' research does not end here, since there are many more important mechanisms to be unravelled. Many exciting avenues of research have been opened by the present work. Dr Francois tells Scientia that they 'are currently cutting these different mobile elements to identify the genes conferring new properties and phenotypes.'

Since S. aureus is a major source of infection for humans and animals, and is evolving into highly infectious and aggressive strains, it is highly important to understand the impact of mobile genetic element acquisition by bacterial genomes. Firstly, these elements produce notable changes in the invasiveness of bacteria, and secondly, the integrity of bacterial genomes has a heavy influence on their future evolution, behaviour, and resistance to antibiotics. Presently, this area of research is still novel and relatively unexplored, which leaves large gaps in our understanding and the ability to create sufficiently targeted antibiotics capable of addressing such infections.

The research led by Dr François next proposes to shed more light on this topic. The team is planning to discover unknown prophages, which confer bacteria important clinical features. By exploring the molecular mechanisms involved in the process that turns bacteria into pathogens, they seek a better understanding of cellular invasiveness. Identifying novel restriction mechanisms encoded by the genetic elements of bacteriophage viruses is another important point on their list, followed by assessing the regulation of the known restriction systems. How these changes become fixed within the genome by DNA methylation processes, and the identification of the exact biological functions encoded in the viral instructions are areas that will be targeted in future research.

'We are currently cutting different mobile elements (mainly composed of hypothetical proteins with unknown functions), to identify the genes conferring new properties and phenotypes'

All these aspects define the virulence, epidemiologic patterns, and pathogenic potential of future staph strains. For this reason, the team aims to decode the instructions carried by the phage genes – instructions which are responsible for the phenotypic changes observed in the latest bacteria generations. This is all the more important due to the increasing number of humanized strains observed in clinical settings.

Meet the researcher

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Dr Patrice François is a team leader at the Genomic Research Laboratory (GRL), a world class research centre in Geneva, where he has been instrumental in its creation and development. In 1996, François received his PhD from Paris XIII University, France, and continued as a post-doc within the Division of Infectious Diseases. His research on the epidemiology and the regulation of virulence factors in S. aureus has attracted multiple awards, such as the GlaxoSmithKline award (2004), the Pfizer award (2004), and the Sanofi-Aventis award (2006). The Faculty of Medicine of Geneva awarded him the Privat-Docent degree for his work on bacterial genome and virulence bases in methicillin-resistant staph, or MRSA. His work includes 181 published research articles and 17 book chapters, totaling a citation index of 49 and bringing him 6,789 citations.

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