Commentary

Big insights from a small outbreak

Nikolai Windbichler

Department of Life Sciences, Imperial College
London, UK

Invited Commentary on 'Parallel bacterial evolution within multiple patients identifies candidate pathogenicity genes', Lieberman et al., Nature Genetics 2011

A small epidemic of *Burkholderia dolosa* broke out among cystic fibrosis patients in Boston in the 1990s in which 39 individuals were infected. Many of the cases were followed up over a period of up to 16 years during which bacterial samples were isolated. Lieberman and colleagues¹ combine this resource with high-throughput sequencing and a modern genomics approach to arrive at a surprisingly diverse set of insights.

First, they sequenced the bacterial genomes from more than a hundred isolates derived from 14 patients and were able to establish the order of transmission events and the phylogeny of *B. dolosa* isolates through the creation of a maximum-likelihood tree. They found that the pathogen had spread from one initial subject to the other patients often passing through multiple subjects. This opened the possibility to study in detail the parallel evolution of this bacterial strain in a clinical setting between and within hosts.

Single nucleotide polymorphisms accumulated in the evolving pathogen genomes at the rate of around two mutations per year, yet the parallel evolution of bacterial pathogens makes it difficult to distinguish truly adaptive mutations from neutral mutations that had become fixed by chance. The authors asked whether some genes had attracted a higher than expected number of mutations arising independently in multiple individuals. They identified a number of bacterial genes in which three or more mutations arose independently in several individuals suggesting that these, in particular, were important for adaptive evolution of the bacteria to the host environment. These genes also showed more non-synonymous mutations than expected, indicating that they were under strong positive selection. The identified genes included some that encode traits known to be disease-related,

such as a bacterial gene associated with fluoroquinolone resistance, as well as genes acting in the membrane synthesis of bacterial O antigens. Others had not previously been involved as disease factors and whereas some had unknown functions, others had a putative role in processes like oxygen-dependent regulation which could indicate a possible link to reports of altered oxygen levels in the mucus of cystic fibrosis patients² and the observation that bacterial virulence can be modulated in response to available oxygen levels.³

The approach taken by Lieberman and colleagues also provides unexpected insights into the mechanism of the bacterial infection. When multiple isolates from a patient's blood were examined, these were often found to derive from different lineages in the patient's lung. Rather than the transmission of a single clone from lungs to blood, this points to either a punctuated transmission of multiple clones from the genetically diverse lung strains or multiple transmissions occurring over time. This analysis thus brings into focus unresolved questions about bacteremia and could ultimately inform therapeutic strategies. Lieberman *et al.* show elegantly how tracking the parallel molecular evolution of a pathogen strain among multiple individuals allows the identification of key pathogenicity genes and can reveal the selective forces acting upon those genes during the infection cycles within the human hosts. The movement of the pathogen within hosts was analyzed in the same way that transmission between hosts was tracked. Systematically identifying selective pressures acting on pathogens within their hosts may ultimately influence the development and choice of therapeutic approaches.

References

- 1 Lieberman TD, Michel JB, Aingaran M, Potter-Bynoe G, Roux D, Davis MR Jr., *et al.* Parallel bacterial evolution within multiple patients identifies candidate pathogenicity genes. *Nat Genet.* 2011;43:1275–80.
- 2 Worlitzsch D, Tarran R, Ulrich M, Schwab U, Cekici A, Meyer KC, *et al.* Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. *J Clin Invest.* 2002;109:317–25.
- 3 Marteyn B, West NP, Browning DF, Cole JA, Shaw JG, Palm F, *et al.* Modulation of *Shigella* virulence in response to available oxygen *in vivo*. *Nature.* 2010;465:355–8.