



Science writer **Meriel Jones** takes a look at some recent papers in SGM journals which highlight new and exciting developments in microbiological research.



Clostridium difficile – a special issue

Clostridium difficile is a bacterial species that lives in oxygen-free conditions. It is one of the very many types of bacteria in the healthy human gut, and although relatively uncommon in adults, can be detected in at least two out of three infants. However, one side-effect of life-saving antibiotic therapies, first recognized in 1977, can be that *C. difficile* multiplies rapidly and causes diarrhoea, which can be extremely serious and life-threatening.

The biology of the bacterium provides resistance to some antibiotics and also allows it to form structures that are highly resistant to disinfectants and other hygiene measures, making it easily spread in places like hospitals. Indeed, it is now the most frequently identified cause of hospital-acquired diarrhoea.

A system called ribotyping to identify strains of *C. difficile* has been devised at the Cardiff Anaerobe Reference Unit, UK, and is now used worldwide. In the last few years, *C. difficile* has become even more notorious because a new, very virulent epidemic strain (NAP1/027)

has emerged in Europe and North America and is responsible for a large increase in both illness and death. At least 27 further very virulent strains have also been identified.

The Second International *C. difficile* Symposium, with delegates from 24 countries in five continents, was held in June 2007 in Maribor in Slovenia. It brought together researchers studying the fundamental biology of this bacterium, clinicians and those focusing on new treatment strategies. The June 2008 issue of *J Med Microbiol* (vol 57, part 6) is devoted to the ideas and information that came from the symposium.

For clinical practice, the way that *C. difficile* is unaffected by the widely used fluoroquinolone antibiotics, used to treat a very wide range of bacterial infections, is very important. The European Study Group on *C. difficile* (ESGCD) reported on the alarming pattern across Europe of increasingly widespread antibiotic resistance. The presence of *C. difficile* disease in farm

animals, recounted by Slovenian researchers, suggested a further undesirable possibility of spread through the food chain. In contrast, researchers from the Alimentary Pharmabiotic Centre in Ireland supplied the novel idea for a therapy using designer probiotics to neutralize both *C. difficile* cells and toxins in the gut.

There is an essential link between the amount of toxins (toxin A and toxin B) produced by *C. difficile* and the severity of the illness. The biosynthesis of the toxins is controlled by a series of positive and negative controls within the cell, in response to the cell's environment. The human gut is stressful for bacteria, and researchers from the UK presented their initial analysis of how all the genes within *C. difficile* react to this situation. Partial loss of control over toxin biosynthesis, as well as resistance to antibiotics, is characteristic of the epidemic strains. French and German researchers told the symposium about studies hinting that antibiotics may actually influence the ability of *C. difficile* to colonize the gut as well as affecting toxin production. In addition *C. difficile* can synthesize, and tolerate, the toxic disinfectant *p*-cresol and researchers from the London School of Hygiene and Tropical Medicine, UK, presented their work looking at whether this provided any competitive advantage. Their study showed that two of the virulent strains tolerated significantly higher levels of *p*-cresol than some others. In addition, researchers in Scotland described their experiments indicating that toxin potency might be affected by additional proteins on the *C. difficile* surface.

The toxins are large, complex, sugar-coated proteins that recognize the surface of human cells, get inside and then wreak havoc in the gut. Researchers at both the Institut Pasteur in Paris, France, and the University of Freiburg, Germany, provided overviews of the current ideas on exactly how these processes happen. The damage is caused because the toxins attach a glucose molecule to a specific point in human proteins that are essential for

transmitting signals within cells. This can kill the cells and researchers from Hannover Medical School in Germany talked about their detailed study of these events. Despite several gaps in our knowledge, the importance of the toxins to illness is very clear and has led to suggestions that a new therapeutic approach would be to develop compounds that block recognition between the toxins and human cells.

The immune system is essential in resistance to bacterial infections and researchers from the University of Edinburgh, UK, showed very clearly that patients with illness caused by *C. difficile* did not differ from apparently healthy people harbouring the bacterium in terms of their immune response. This is good news for proposals to develop treatments, including vaccines, that rely on the immune system, recounted by scientists from Italy, Ireland and the UK.

◀ Coloured SEM of *C. difficile* on a surface.
Annie Cavanagh and Dave McCarthy/
Wellcome Images

Researchers from around the world shared information about the prevalence of *C. difficile* in hospitals within their own countries. For example, scientists from the Netherlands explained how the realization that NAP1/027 strains had caused epidemics in eight Dutch hospitals in 2005 led rapidly to new hygiene guidelines and a review of the use of antibiotics prior to each outbreak. Staff from the Austrian Agency for Health and Food Safety had checked 149 samples from Austrian hospital laboratories in 2006 to discover what ribotypes were present and whether they differed in lethality. They identified 41 different ribotypes and pointed out that finding several infections with the same ribotype in a hospital should prompt an in-depth investigation in case the source was in the hospital itself. Twelve of the patients had died, infected with six different ribotypes, illustrating that there is serious danger from more than the NAP1/027 strain.

The experience of Korean researchers between 2000 and 2005 made the point

that some epidemic strains lack toxin A. The prevalence of these strains has been between 0.2 and 56 % in reports from around the world. Unfortunately, some tests rely on detecting this toxin so that the infection is misdiagnosed, emphasizing the importance of developing appropriate routine identification methods. In addition, work from the research group at the London School of Hygiene and Tropical Medicine, UK, indicated that the toxin situation was becoming even more complex since some virulent strains have novel versions of toxin B.

At the end of the symposium, the delegates agreed what the future research priorities should be. Advances have been made on several since June 2007, and they include the areas of epidemiology, establishment of better nomenclature and typing systems for isolates, and research to gain greater understanding of both pathogenesis of the bacterium and the reasons for susceptibility of its unwilling human hosts.

Pig gut bug

Baele, M., Decostere, A., Vandamme, P., Ceelen, L., Hellemans, A., Mast, J., Chiers, K., Ducatelle, R. & Haesebrouck, F. (2008). Isolation and characterization of *Helicobacter suis* sp. nov. from pig stomachs. *Int J Syst Evol Microbiol* **58**, 1350–1358.

The bacterial species *Helicobacter pylori* lives in the stomachs of about half the human population and is involved in causing stomach ulcers. Its spiral-shaped cells inhabit the mucus lining of the highly acidic stomach. Researchers have also detected other helicobacters in some people, distinguished by their more tightly coiled spiral-shaped cells and differences in gene sequences. These bacteria also have an association with stomach ulcers and lymphoma. They have been provisionally named *Helicobacter heilmannii*, although there seems to be more than one type. Unfortunately, it has proved impossible to grow *H. heilmannii* type 1 as a pure culture in the laboratory, hindering further investigation.

Helicobacter species also live in animal stomachs. For example, DNA from 'Candidatus *Helicobacter suis*' can be detected in at least 60 % of all pigs. Although these bacteria were first observed around 1990, they also have never been isolated or studied in detail. Researchers at Ghent University in Belgium became interested because, in addition to a potential role in animal welfare as the cause

of ulcers and gastritis in pigs, the only piece of molecular genetic information matched some helicobacters from people suspiciously well. There was also epidemiological evidence that people who had contact with pigs had a higher risk of *H. heilmannii* infection, suggesting that the bacteria might transfer from pigs to humans.

To resolve the identity of these pig bacteria, the Belgian researchers went to great lengths to devise a way to grow them in the laboratory. Finally, starting from the mucus scraped from pig's stomachs that had been soaked in dilute acid, they were able to obtain cultures. The special growth medium they devised contained a complex mixture of components, including activated charcoal, vitamins and several antibiotics maintained in an atmosphere that was very low in oxygen and high in carbon dioxide. Obtaining a quantity of pure 'Candidatus *Helicobacter suis*' allowed them, for the first time, to perform a battery of cultural and molecular tests on the bacteria.

The results made it very clear that the bacteria from pigs belong to the same species as type 1 strains of *H. heilmannii* from people, and are different from any other well-characterized species of *Helicobacter*. To resolve the question of what to call it, the researchers have proposed that *Helicobacter suis* is used from now on.



Fat target for TB treatment

▲ Coloured TEM of *M. tuberculosis*.
Kwangshin Kim / Science Photo Library

Bhowruth, V., Brown, A.K. & Besra, G.S.

(2008). Synthesis and biological evaluation of NAS-21 and NAS-91 analogues as potential inhibitors of the mycobacterial FAS-II dehydratase enzyme Rv0636. *Microbiology* **154**, 1866–1875.

Tuberculosis is making an unwanted return around the world. Worryingly, some new strains are resistant to most or even all of the therapeutic drugs. Isoniazid is the current mainstay in treatments. It interferes with the synthesis of mycolic acids that form an essential, fat-rich permeability barrier around the *Mycobacterium tuberculosis* cell. Researchers would like to find more drugs that affect this process to provide treatments for the new drug-resistant cases of TB.

Mycolic acid biosynthesis involves two enzyme complexes and the second one, FAS-II, only occurs in bacteria, plants and some parasites. The enzymes take long-chain fatty acids and make them even longer, adding some chemical modifications along the way. Some of the steps are unique to *Mycobacterium* species. Although the genome of *M. tuberculosis* has been sequenced, researchers are still not quite sure what some of the genes do. In addition, although they understand the process that makes mycolic acids, there is still a debate about exactly which genes in *M. tuberculosis* provide the instructions for each step. A key step is dehydration and the gene Rv0636 is a strong candidate for control of the process. Finding chemical compounds that inhibit this step could solve the identity of the gene and suggest new therapies.

Researchers at the University of Birmingham, UK, are looking for new anti-TB drugs. They have tested the effects of two series of chemicals that should inhibit the dehydration enzyme activity. In each case, they started with a chemical that was already known to inhibit the enzyme, made a small modification to this chemical and then tested the new, pure chemical for its effect on both the growth of *Mycobacterium* cells and FAS-II enzyme activity. In addition to normal *Mycobacterium* cells, they also tested cells that produced high levels of the Rv0636 gene product.

Some of their modifications to a chemical called NAS-21 were very effective against the *Mycobacterium* cells, and cells with lots of the Rv0636 gene product were particularly resistant. Some changes to a second chemical, NAS-91, also made it more toxic to the cells. When the researchers tested the effect of the chemicals on an authentic FAS-II enzyme, the chemicals that had most effect on the bacterial cells also caused most inhibition of the enzyme. The coincidence of these results is a strong indication that Rv0636 is the gene for the essential dehydratase. The chemicals themselves may be candidates for further development towards new drugs that may eventually be useful for treating TB.

Maddeningly elusive

Julius, C., Heikenwalder, M., Schwarz, P., Marcel, A., Karin, M., Prinz, M., Pasparakis, M. & Aguzzi, A. (2008). Prion propagation in mice lacking central nervous system NF- κ B signalling. *J Gen Virol* **89**, 1545–1550.

The defining feature of transmissible spongiform encephalopathies like BSE and scrapie is that a mis-folded prion protein accumulates in the brain and the appearance of the brain tissue changes as cells die and gaps form. The result is distressing changes in behaviour and invariably death. Scientists have no idea how the abnormal protein causes these dramatic effects. However, there are well-known physiological systems, including the NF- κ B signalling pathways, that carry signals for inflammation and regulate cell death which are activated in other neurodegenerative diseases like Alzheimer's and Parkinson's diseases. Researchers have conflicting evidence for the role of this pathway in BSE. In principle, NF- κ B signalling could be beneficial or deleterious, depending on the consequences of the signals. These could enhance the survival of neurons by inducing processes that counter cell death, or promote their death through the release of toxic molecules.

An international research effort, led by Adriano Aguzzi in Switzerland, has now taken the approach of recording the progress of prion disease in mice that lack components of the NF- κ B pathway. One set of mice lacked a component for pro-inflammatory signalling in most brain cells while others lacked part of an alternative NF- κ B signalling pathway throughout their bodies. Surprisingly, the researchers could not detect any difference between the symptoms and progress of the disease in these and normal mice.

The implication is that the NF- κ B pathways are not important in prion disease, despite their importance in regulating the immune response and thus in processes such as cancer and autoimmune diseases. The mechanisms for loss of brain cells during BSE continue to be elusive.