

## *Clostridium difficile*

In 1935, Hall and O'Toole recognized a previously undescribed anaerobic bacterium in the stools of newborns and named it *Bacillus difficilis* (Hall & O'Toole, 1935). Almost 40 years later, following an increase in the rare but dangerous pseudomembranous colitis (PMC), linked to antibiotic use, the species now known as *Clostridium difficile* was identified as the aetiological agent of antibiotic-associated PMC (Bartlett *et al.*, 1978). Over the next three decades, hundreds of studies have been published and several monographs written on the bacterium and the spectrum of diseases with which it is associated. Though some specialized meetings had been held earlier, it was not until 2004 that the first international *Clostridium difficile* symposium (FICDS) was held. This took place between 5 and 7 May 2004 in the beautiful if rather damp surroundings of the Julian Alps in Kranjska Gora, Slovenia. Over 90 delegates from 19 different countries attended. Many of the major players involved in *C. difficile* research in the past quarter century were present.

The symposium brought together invited speakers whose task was to bring the attendees up to date by describing work, much of which had been published. All authors of offered papers (oral and poster) together with any of the invited speakers who had presented previously unpublished work were invited to submit manuscripts for this special issue of the *Journal of Medical Microbiology*. All submitted papers were subject to the usual editorial process. This special edition contains the papers that were accepted.

Perhaps the most important of these papers is the 'Revised nomenclature of *Clostridium difficile* toxins and associated genes' by Rupnik *et al.* (2005). This multi-authored paper was the culmination of a round-table session where most of the key players in the field were present. The first draft was sent out to other key individuals who had been unable to attend FICDS to ensure that what is reported here is a consensus view.

The main symposium consisted of an introductory session, followed by sections

on genetics, physiology, toxins, diagnosis and epidemiology, pathogenesis, and prevention and treatment. Peter Borriello kicked off the proceedings by describing the historical aspects of *C. difficile* and the diseases it causes. This demonstrated Peter's close involvement with so many of the seminal studies of the organism – from the very early days, Larson *et al.* (1978), through three decades (e.g. Borriello, 1990) to the present time (Johal *et al.*, 2004). Dale Gerding, who along with Stuart Johnson and colleagues has been one of the leading clinical investigators of the disease (e.g. Johnson & Gerding, 1998), introduced the organism in its clinical setting. Glen Songer completed the introductory session by describing the importance of *C. difficile* in domestic animals, and introduced some new data to show that it may be an important cause of neonatal enteritis in pigs.

The section on 'Genetics' began with Brendan Wren reporting on the *C. difficile* genome project. This is now complete and can be accessed at [http://www.sanger.ac.uk/Projects/C\\_difficile/](http://www.sanger.ac.uk/Projects/C_difficile/). The culmination of this massive project will no doubt spawn many future studies in pathogenesis. Peter Mullany reviewed mobile genetic elements in *C. difficile*, and some of his group's new work is presented in this issue (Hussain *et al.*, 2005). Julian Rood completed the session by describing the hard-fought and continuing struggle to develop systems for the genetic manipulation of *C. difficile*, which is appropriately named 'difficult'. Linc Sonenshein began the next section on 'Physiology' with a discussion on stationary phase and sporulation. He demonstrated the practical significance of the spore as the reservoir of disease and showed how synthesis of the major toxins is restricted to bacteria that have made the transition from exponential phase to stationary phase and sporulation. Quorum sensing is important in many bacterial pathogens, and this cell-to-cell communication is a potential target for control of disease. Nigel Minton described some new work on quorum sensing in *C. difficile* showing the presence of an autoinducer 2 (AI-2)-like molecule

and a putative negative regulator of AI-2. The relevance of these to toxin production or any other putative pathogenic mechanism is, however, not yet known (Carter *et al.*, 2005).

Understandably, the next session on 'Toxins' covered some of the most exciting and well-worked areas of *C. difficile* research. Christoph von Eichel-Streiber described the pathogenicity locus of *C. difficile*, and this was followed by Ingo Just covering the mode of action of toxins A and B and their use as tools in cell biology. Bruno Dupuy followed this with a discussion on the regulation of toxins. It is well known that many bacterial toxins are encoded on transferable genetic elements such as plasmids and bacteriophage. Is this the case for *C. difficile*? Some novel work by Goh *et al.* (2005) investigated several temperate bacteriophages from clinical isolates of *C. difficile*. When a non-toxin producer was lysogenized with any of these phages, none was converted to being able to produce toxin. However, three of the phages carried one of the accessory toxin genes, *tcdE*: some of the lysogens carrying these phages appeared to produce significantly more toxin B. Regulation may therefore be under the control of phage-encoded genes. Michel Popoff rounded off the session with a presentation on ADP-ribosyltransferase and clostridial binary toxins. This topic was also covered in the offered papers, and two studies are included in this issue (Barbut *et al.*, 2005; Pituch *et al.*, 2005). The real significance of the binary toxin in *C. difficile* is not yet understood.

From the point of view of both patient and clinician, the diagnosis and epidemiology of *C. difficile* infections are probably the most important aspects of *C. difficile*-associated disease (CDAD) research. Since the development of the selective CCFA medium by George *et al.* (1979), the isolation of *C. difficile* has been easy. When *C. difficile* was being recognized as a major pathogen in the early 1990s, most laboratories began culturing all diarrhoeal stool specimens from hospitalized patients for the organism. However, many clinicians did not know the significance of a 'toxin'-

negative isolate: it was therefore thought more appropriate just to test for toxin. 'Toxin' detection, initially by tissue culture assay for toxin B, and more recently by immunoassays for toxin A and/or B, had always been part of the diagnostic protocol, but with the advent of rapid tests to detect 'faecal toxin' and coincidentally a reduction in staff levels in many centres because of financial restraints, there has been a trend away from culture and isolation of *C. difficile*. In recent years, following the development of rapid toxin A+B kits, culture of the organism has been discontinued entirely by many laboratories, certainly throughout Europe (Barbut *et al.*, 2003). In his invited paper, Michel Delmée described the current trends, but showed the real need for culture of the organism, and not just from the point of determining sensitivity to antibiotics and being able to investigate outbreaks by typing isolates. There is a real chance that many cases of CDAD may be missed if toxin-negative stools from symptomatic patients are not cultured. In this Belgian study described here, where over 10 000 diarrhoeal stool samples were investigated, the number of positive diagnoses could be increased by approximately 75 % if culture of stools was done from all symptomatic patients. If any *C. difficile* isolates arose from toxin-negative stools, the colonies were tested for toxin production. Many of these turned out to be toxin producers and were considered to be involved in the pathogenesis of the disease (Delmée *et al.*, 2005). The subtitle of the paper is apt: 'a plea for culture'.

Jon Brazier described the various typing methods available for *C. difficile* – a field of much debate. Since the organism was first shown conclusively to be a true nosocomial pathogen by various typing and 'fingerprinting' techniques (Poxton *et al.*, 1984; Tabaqchali *et al.*, 1984), such studies have been a popular pursuit over the years – but with little standardization. Tom Riley concluded the section with a review of the epidemiology of CDAD. He drew attention to the importance of the costs of managing cases of CDAD, especially where relapses occur. In this special issue, several papers are related to this section of the symposium. They include a study that reinforces the important message that it is prudent to type more than a single isolate from a faecal sample, especially when investigating relapses or outbreaks (van den Berg *et al.*, 2005): it is common to have multiple strains

in an outbreak of *C. difficile*. Although S-layer typing has been used as an easy and useful typing method (McCoubrey *et al.*, 2003), a modification of this is proposed for genotyping isolates by sequencing the variable region of the *slpA* gene – the gene encoding the surface layer proteins (Kato *et al.*, 2005). Whether it is easier to do a phenotypic or a genetic method probably depends on the experience and equipment of the individual laboratory. PFGE is the standard method for genotyping many different bacterial pathogens, but it has not been used extensively with *C. difficile*, largely because so many strains have been considered non-typable because of degradation of the DNA. Alonso *et al.* (2005a) have modified the procedure to permit 100 % typeability of their isolates. Transmission of *C. difficile* from animals to humans has often been considered and Arroyo *et al.* (2005) used PCR ribotyping to explore this further. They investigated isolates mainly from humans, horses and dogs and found the same ribotypes common to more than one species, but different ribotypes predominated in different species.

The penultimate section on 'Pathogenesis' was begun by the author of this editorial (Ian Poxton), who described our current state of knowledge of this field, which includes some of the best understood aspects of *C. difficile* research, the action of toxins at the cellular level, and the least understood, the role of the host response. An offered paper by Péchiné *et al.* (2005) showed that patients with CDAD produced antibodies to several surface proteins which are putative adhesins. From their results, the authors suggest that an immune response to adhesins may play a role in host defence. The concept of vaccination and/or immunotherapy was introduced by Larisa Miller, who gave an update on the new vaccine being developed by Acambis. Rather than active vaccination of at-risk patients, the vaccine may be used for producing immunoglobulin prepared from vaccinated human volunteers for passive administration. Another approach to passive immunotherapy was described by van Dissel *et al.* (2005). Here cattle were immunized with inactivated toxins and killed whole cells of *C. difficile*. The whey prepared from their milk is high in specific secretory immunoglobulins and these antibodies neutralize the cytotoxin *in vitro* and protect hamsters from experimental infection. In a small-scale human study,

none of nine patients with a history of relapse suffered further relapse after they had whey therapy following standard antibiotic treatment.

The final section covered the prevention of CDAD in the hospital by Mark Wilcox, the current state of antibiotics and resistance in *C. difficile* by Paola Mastrantonio, and finally alternative (mainly probiotic) treatments for *C. difficile* by Lynne McFarland, which are reviewed in this issue (McFarland, 2005). Offered papers related to this section included the results of a study of over 200 isolates from a Spanish teaching hospital which showed that 5 % of the isolates were of the A<sup>-</sup>B<sup>+</sup> phenotype (Alonso *et al.*, 2005b). Ackermann *et al.* (2005) reported on a 10-month study on the bacteriology of patients with antibiotic-associated diarrhoea, and raised questions as to the relevance of *Clostridium perfringens* and *Staphylococcus aureus* in the aetiology of the problem.

On a personal view, I suggest that the major areas of research on *C. difficile* in the next few years should be directed towards the many unanswered questions – some of which are listed below:

- Are there differences in virulence between A<sup>+</sup>B<sup>+</sup> and A<sup>-</sup>B<sup>+</sup> strains, and does the binary toxin have a significant role to play in pathogenesis?
- Why are some types much more virulent than others?
- Is resistance to either the precipitating antibiotics or those used for treatment a problem?
- Do the toxins have any role in the healthy intestine, for example in infants?
- What makes patients susceptible?
- Is intravenous immunoglobulin a worthwhile treatment?
- Is immunity to cell surface components important in protection and is there a role for whole cell or subunit vaccines?
- Is it possible to measure mucosal immunity and is the mucosal arm crucial for protection?
- Does *C. difficile* possess mechanisms for evasion of the immune response?
- Is there a physiological or immunological reason for the existence of so many different S-types?
- Have 'super strains' evolved?

Since the symposium ended, news has broken of a major outbreak of CDAD in Quebec, Canada. Reports were broadcast all

over the world and the local Canadian Broadcasting Corporation (CBC) news announced 7000 sufferers and 600 deaths in Quebec, which are four times and six times the expected levels, respectively (CBC website, [http://www.cbc.ca/story/canada/national/2004/10/20/c\\_difficile041020.html](http://www.cbc.ca/story/canada/national/2004/10/20/c_difficile041020.html), accessed November 2004). The apparent increased severity of the disease in Canada has already suggested a strain of increased virulence (Pepin *et al.*, 2004).

In summary, *C. difficile* is an organism of increasing importance, especially in the elderly in the hospital setting. Although its incidence may be controlled somewhat by careful antibiotic prescribing, it is not going to disappear. Novel methods, whether chemical, probiotic or immunological, must be developed to control the organism and the diseases it causes. To help us do this we need to understand in much more detail the interplay between the pathogen and the host in health and disease.

The symposium was organized under the auspices of the European Society for Clinical Microbiology and Infectious Disease (ESCMID) and the ESCMID study group for *Clostridium difficile* (ESGCD). The ESGCD web page can be found at <http://www.escmid.org/esgcd>, where there are links to FICDS and other *C. difficile* resources.

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