

## ***CLOSTRIDIUM DIFFICILE* - EMERGING IN AUSTRALIA?**

Lyn Gilbert

**Background:** *Clostridium difficile* was first described in 1935 – as *Bacillus difficilis* – and identified in 1978 as the commonest cause of antibiotic associated diarrhoea and pseudomembranous colitis. It is often acquired in healthcare settings. Although it is not commonly found in the gastrointestinal tract of healthy adults (2-5%), up to two thirds of young children are colonised prior to weaning (1). Acquisition of *C. difficile* does not always cause diarrhoea, but alteration of commensal bowel flora by antibiotics, proton pump inhibitors, cytotoxic drugs or immune suppression can lead to overgrowth of and toxin production by *C. difficile*. *C. difficile* infection (CDI) is most common in elderly hospital patients or nursing home residents, particularly after a course of broad-spectrum antibiotics and in patients with other underlying disease or following gastrointestinal surgery.

**Bacteriology:** *C. difficile* is a spore-forming anaerobic Gram positive rod, which produces two major toxins - A (enterotoxin) and B (cytotoxin) - that are important in disease pathogenesis. Many colonising strains produce neither, but most pathogenic strains produce both; in Australia, about 10% produce only toxin B. A small proportion of isolates produce “binary” toxin, which apparently complements toxins A and B and is associated with increased virulence. Toxins A and B are encoded by the genes *tcdA* and *tcdB*, respectively, which are part of the pathogenicity locus (PaLoc). The PaLoc also contains *tcdR* and *tcdE*, which regulate toxin secretion and *tcdC*, a negative regulator of toxin synthesis.

**Epidemiology:** In the past 10 years, *C. difficile* has caused increasing concern, with the emergence of a highly virulent epidemic strain which can spread rapidly and cause relatively severe disease with significant mortality (5-10%) (2) especially, but not exclusively, in people over 65. It was initially identified in

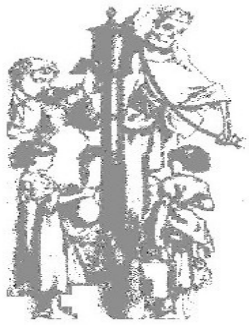
North America but rapidly spread to United Kingdom and many European countries (3). Between 1991 and 2003 at a Canadian University Hospital, the incidence of CDI among patients over 65 increased from 102/100,000 to 867/100,000 population and the proportion of complicated cases, from 7.1% to 18.2% (4).

The *C. difficile* strain, which is largely responsible for the increased incidence and severity of CDI in the northern hemisphere, is variously described, depending on the molecular typing method used, as: group BI (restriction endonuclease analysis); type NAP1 (pulse-field gel electrophoresis); type 027 (PCR ribotyping). It has an 18bp deletion, which modifies the repressor effect of *tcdC*, leading to increased toxin A and B production (5); it also produces binary toxin (6) and is resistant to fluoroquinolone antibiotics, which facilitates its spread in settings where these agents are widely used.

**CDI in Australia.** Until recently there has been no evidence of dissemination of *C. difficile* 027 in Australia (although there is very limited surveillance data). A single imported case of 027 CDI was reported in 2008 (7). However, recently a small outbreak of locally transmitted cases at a Melbourne hospital, has caused increased concern about possible dissemination of this strain in Australia and renewed calls for: better surveillance (case reporting and selected strain typing); continued improvements in infection control and antibiotic stewardship and more improvements in diagnostic testing for *C. difficile*, in patients with diarrhoea. Apart from increased morbidity and mortality and high rates of recurrence of CDI due to hypervirulent strains, the estimated excess cost of a single case is US\$5,000-8,000 for primary, and ~US\$14,000 for recurrent, disease (8). This makes investment in prevention, including improved surveillance, infection control and antibiotic stewardship.

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**Prevention:** Australian Health Ministers have recently agreed that reporting of CDI by hospitals should be mandatory. The Australian Commission on Safety and Quality in Healthcare (ACSQH), has initiated major programs to improve hand hygiene and antibiotic stewardship throughout Australian hospitals which, if fully implemented, will significantly reduce the risk of transmission of *C. difficile*.

*C. difficile* spores can become widely distributed in the environment and persist for long periods. Infection can spread by contact with contaminated objects or hands of healthcare workers. Hand washing with soap and water is more effective than alcohol-based hand rub (ABHR - now widely promoted for hand hygiene), in removing spores but appropriate use of contact precautions and adequate environmental cleaning should limit hand contamination. The use of ABHR, according to Hand Hygiene Australia's (<http://www.hha.org.au/>) "5 moments" program, is recommended unless hands are visibly soiled.

Antibiotic therapy is the main risk factor for CDI, so appropriate use is essential for prevention. Antibiotics should not be used unnecessarily or for longer than required and broad spectrum agents, such as ampicillin, 3rd or 4th generation cephalosporins, clindamycin and the newer fluoroquinolones (moxifloxacin and gatifloxacin) should be avoided if possible (9); the latter are strongly implicated in dissemination of *C. difficile* 027 (10).

**Diagnosis:** Most laboratories use rapid enzyme immunoassays (EIA) for toxins (A or A plus B). It is now clear that most toxin EIAs have relatively poor sensitivity, specificity and predictive value (PVs). An EIA for the *C. difficile*-specific enzyme, glutamate dehydrogenase (GDH) is sensitive (high negative PV) but detects non-toxicogenic strains, with ~30% false positive rate, and requires confirmatory testing. "Gold standard" diagnostic methods are: a) toxicogenic culture (i.e. culture, on selective media following "alcohol shock" treatment of the specimens to reduce vegetative faecal bacterial contaminants), followed by toxin test) or b) cell culture cytotoxicity assay (CCA i.e. cytotoxicity of a faecal extract for cell monolayers). (11, 12).

Both are relatively slow ( $\geq 48$  hours) and CCA is technically difficult, poorly standardized, and detects only toxin B. In-house and commercial PCR assays, (targeting one or more toxin e.g. *tcdB* or toxin regulatory genes in the PaLoc) have not been widely evaluated but limited evidence suggests that they are highly sensitive and specific although commercial assays are expensive. If surveillance is to be improved, some laboratories will need to perform cultures e.g. on EIA or PCR positive specimens, so that molecular typing can be performed.

**Indications for testing:** Patients who develop diarrhoea >48 hours after hospital admission or are admitted with antibiotic-associated diarrhoea should be tested by a rapid test to ensure appropriate treatment and infection control. Less sensitive assays require confirmation (e.g. GDH plus toxin EIAs followed by toxicogenic culture of GDH positive specimens or PCR alone). *C. difficile* diagnostic tests should not be performed on formed stools and the yield is not increased by testing multiple specimens (15).

Culture should be performed routinely in patients with severe CDI as indicated by some or all of fever ( $>38.5^{\circ}\text{C}$ ), acute abdomen with ileus and/or toxic megacolon (based on clinical, radiological and colonoscopic examination), hypotension, elevated white cell count, low albumin and acute renal impairment.

**Management:** The antibiotic of choice for mild to moderate disease is oral metronidazole. Vancomycin is recommended for severe disease only (to minimise the risk of vancomycin resistant enterococci emerging) and is associated with lower rates of treatment failure and relapse than metronidazole, particularly in severe disease (14, 15). Since recolonisation from the environment is thought to be the main cause of recurrence and resistance to metronidazole or vancomycin is rare in Australia (16), routine treatment of first recurrences is as for primary disease.

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**Professor Agus Purwadianto & Professor Tania Sorrell**



**Professor Bruce Robinson & The Honourable Carmel Tebbutt**

## **SEIB Inaugural Conference**

The 18 May 2010, marked the launch of Sydney's newest institute, the Institute for Emerging Infectious Diseases and Biosecurity (SEIB), at the University of Sydney. Deputy Premier and Minister for Health for New South Wales, the Honourable Carmel Tebbutt, officially opened the inaugural conference 'SEIB 2010'.

SEIB is a major new multidisciplinary hub on the Westmead and Camperdown campuses of the University of Sydney, dedicated to research, capacity building, advocacy and communication in the Asia Pacific region. It was established in February 2010 in response to increasing threats posed to humans and animals by emerging and re-emerging infectious diseases including HIV, avian and H1N1 influenza, SARS, Hendra virus infection, drug resistant TB and other increasingly antibiotic resistant infections.

The two day conference was the first step in the development of SEIB, and a great opportunity for academics, health, veterinary, law and other professionals and students to listen and learn from a variety of individuals with many areas of expertise and experience. Professor Tony McMichael, Dr Martyn Jeggo and Ms Julie Robotham shared their expertise in epidemiology and the effects of climate change on emerging infectious diseases, animal health and biosecurity, and journalism, respectively. The presence of Professor Agus Purwadianto, the Director General of the National Institute of Health, Research and Development, Ministry of Health Indonesia, Dr Pretty M Sasono, the Head of the National Laboratory of Infectious Diseases Research at the National Institute of Health, Research and Development, Ministry of Health Indonesia, and Dr Nurjati C. Siregar, Research Manager FKUI, University of Indonesia is a true testament to the spirit of collaboration and partnership between institutions.



## **Staff Profile .....**



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Trang Nguyen joined ICPMR-CDMLS in 2009 after working in private pathology laboratories in Australia and New Zealand. She gained a bachelor of medical laboratory science in 2001, majoring in medical microbiology and clinical biochemistry from the University of Otago, New Zealand. Whilst working for private pathology, Trang worked in general Microbiology where she gained experience from reading microscopy to culture interpretation of different clinical samples.

Since joining ICPMR-CDMLS, Trang has worked in Molecular biology using molecular techniques in conducting single, nested, ELISA and real-time (RT)-PCR and 16S rDNA sequencing assays on clinical isolates. Currently Trang is working in the reference laboratory section of CIDMLS-ICPMR. She is rotated within different sections of the reference laboratory ie molecular biology, PC4 lab and Mycobacterium reference lab. At the moment she's working in the Mycobacterium reference laboratory where she helps perform sensitivities for *Mycobacterium tuberculosis* and identify different *Mycobacterium* spp. using different methods including culture, high performance liquid chromatography, PCR, and 16S rDNA sequencing.

Coming from a background of traditional culture-based microbiology, Trang has now gained experience with molecular techniques. She is working on developing a multi-locus variable number tandem repeat analysis (MLVA) assay for *Clostridium difficile* typing with Dr Qinning Wang.

## **Upcoming events....**

### **SEIB & CIDM-PH Emerging Infectious Disease Clostridium Difficile Symposium**

**Are we prepared for the virulent "Quebec" strain?**

**Westmead Hospital, Thursday 5<sup>th</sup> August 2010**

A symposium on *Clostridium difficile* infection presented by the Westmead Association Hospital Week, Sydney Institute for Emerging Infectious Diseases and Biosecurity (SEIB) and Centre for Infectious Diseases and Microbiology – Public Health. *Speakers include:*

- Professor Tania Sorrell
- Dr Vu Kwan
- Professor Lyn Gilbert
- A/Professor Jon Iredell
- Dr David Mitchell
- Ms Kathy Dempsey
- Ms Jo Tallon

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