Epidemic potential and antimicrobial resistance in *Clostridium difficile*

**Thomas V Riley**  
Microbiology & Immunology  
The University of Western Australia  
Queen Elizabeth II Medical Centre  
Nedlands 6009 Western Australia  
And  
Division of Microbiology & Infectious Diseases  
PathWest Laboratory Medicine  
Queen Elizabeth II Medical Centre  
Nedlands 6009 Western Australia  
Tel: 61 8 9346 3690  
Fax: 61 8 9346 2912  
Email: triley@cyllene.uwa.edu.au

*Clostridium difficile* is the most commonly diagnosed cause of infectious hospital-acquired diarrhoea. *C. difficile* was first isolated in 1935 but not identified as the main causative agent of antibiotic-associated diarrhoea (AAD) and pseudomembranous colitis (PMC) (Figure 1) until 1977. The spectrum of disease caused by *C. difficile* ranges from asymptomatic colonisation to colitis that can progress to more severe PMC. Complications include colonic perforation and death. The term *C. difficile*-associated diarrhoea (CDAD) is used to describe the symptomatic manifestations of the disease, thus excluding asymptomatic colonisation. Hospital inpatients with CDAD are generally elderly and have several comorbid conditions. The majority of these patients have been exposed to antimicrobials that reduce 'colonisation resistance' of the large intestine allowing subsequent infection with *C. difficile*. Whether infection progresses to disease is determined by many factors such as antibiotic exposure, age and comorbidities, and others that are as yet unknown. Acquisition of *C. difficile* is facilitated by its ability to form spores (Figure 2) that are resistant to many disinfectants, allowing it to remain viable in the hospital environment for long periods of time. Toxigenic isolates of *C. difficile* usually produce two toxins, toxin A and toxin B, and these are thought of as the major virulence factors. CDAD is a major financial burden on healthcare systems, with patients spending an extra one–three weeks in hospital costing US$5–10,000 per episode.

**Epidemic C. difficile**

There is now great concern worldwide about CDAD, following the recent emergence in Canada, the USA, and now Europe of a highly virulent strain of *C. difficile* (called PCR ribotype 027 in Europe and NAP1 in the USA). Rates of detection of *C. difficile* have risen dramatically, the *C. difficile* disease has been more severe, and attributable mortality was >10% in those aged >60 years. There has been much discussion on what might be driving this epidemic of CDAD in various parts of the world and the likelihood of the epidemic strain becoming established in Australia. This discussion has focused on several areas, including the role of gastric acid suppressants, the role of animal reservoirs, the emergence of a more virulent strain and the development of antimicrobial resistance.

One possible novel risk factor is exposure to gastric acid suppressants such as histamine-2 receptor inhibitors or proton pump inhibitors. These agents have been more commonly prescribed in recent years and may be associated with increased rates of CDAD in the community, although some case-control studies with hospital patients show no association. Since its
discovery *C. difficile* has been associated with animals. Early investigations showed, for example, that 40% of cats and dogs attending a veterinary clinic harbour *C. difficile*; however, there was no relationship with human strains [15]. More recently, *C. difficile* has been shown to be associated with diarrhoeal diseases in pigs [13], cattle [14] and horses [15]. Rupnik [16] has recently suggested the possibility of *C. difficile* being part of a zoonosis.

In addition to toxins A and B, some strains of *C. difficile* produce a binary toxin (actin-specific ADP-ribosyltransferase, CDT), first reported in 1988 but not considered important until now [17]. Binary toxin producers make up the majority of strains isolated in the large outbreaks of disease overseas [18] and binary toxin has been thought of as an additional virulence factor. Barbut et al. [17] show a correlation between binary toxin production and severity of diarrhoea, and more community-acquired CDAD was caused by binary toxin producers. However, the significance of binary toxin clearly needs further investigation as strains of *C. difficile* that produce binary toxin only, and no toxin A or B, do not cause disease in the hamster model [18].

**Antimicrobial resistance**

While all of the above is true, the most likely driver of the current epidemic is antimicrobial resistance. *C. difficile* isolates are typically susceptible in vitro to metronidazole and vancomycin, and show variable susceptibility to other antimicrobial agents. The relative risk of CDAD following antimicrobial therapy increases if the strain of *C. difficile* is resistant to the antimicrobial [19]. Resistance to various antimicrobial agents appears to be increasing [20]. In Spain, the prevalence of metronidazole resistance was 6.3% in a study of 415 isolates of *C. difficile* and, although full resistance to vancomycin was not found, the prevalence of intermediate resistance was 3.1% [21]. Another study in an Israeli hospital found the prevalence of metronidazole resistance was 2% [22]. The mechanism of metronidazole resistance is unknown, but resistance is frequently associated with inefficient activation of the drug [23].

Resistance to macrolide-lincosamide-streptogramin (MLS) agents such as erythromycin or clindamycin is mediated by a 23S ribosomal RNA methylase encoded by a group of homologous *erm* genes [24]. An investigation of outbreaks that occurred in four hospitals in the USA between 1992 and 1998 found a new clindamycin-resistant strain to be responsible [25]. More recently, a clindamycin-resistant toxin A/B+ strain was found to be widely disseminated in two hospitals in Warsaw, Poland [26]. In a study at the Royal Hospital in Edinburgh, Scotland, clindamycin resistance was extremely common, with only 8% of clinical isolates susceptible [26].

The resistance traits described above have been known about for some years. However, it is resistance to the newer fluoroquinolone antimicrobials that is thought to be playing a major role in the emergence and spread of the PCR ribotype 027 strain responsible for the epidemics in North America and Europe, and excessive fluoroquinolone use appears to be a contributing factor in these recent outbreaks [27]. The problem of fluoroquinolone resistant *C. difficile* was first brought to our attention in 2004 with a report of an outbreak at a long-term care facility in the USA [28]. In an accompanying editorial, Dale Gerding correctly identified that this was an issue of previously uncommon fluoroquinolone resistance in *C. difficile*, while the gastrointestinal tract anaerobe flora remained susceptible [29]. *C. difficile* develops resistance to fluoroquinolones soon after exposure. Ackermann et al. [29] describe 33 moxifloxacin resistant toxigenic isolates (MIC ≥16 mg/L) where resistance was significantly associated with prior quinolone exposure and mutations in the gyrA gene.

All the newer fluoroquinolones including gatifloxacin and levofloxacin and, somewhat surprisingly, the older ciprofloxacin have been implicated [27]. Ciprofloxacin has always been thought of as a low risk antimicrobial for inciting CDAD [27]; however, once *C. difficile* becomes resistant to the later fluoroquinolones it is also resistant to ciprofloxacin and the resistance trait may become more important for initiation of disease. Both gyrA and gyrB mutations have now been implicated in fluoroquinolone resistance in *C. difficile* [31]. The current outbreak strain of *C. difficile* (PCR ribotype 027) has been recognised for over twenty years, but older strains of the same ribotype are susceptible to fluoroquinolones. It is likely that increasing fluoroquinolone usage is acting as a selective pressure, prompting the emergence of this strain. Two other common strains in UK hospitals, PCR ribotypes 001 and 006, have significantly greater resistance to erythromycin, imipenem and levofloxacin [27]. This resistance may
give them a selective advantage over other *Clostridium difficile* strains, and explain their high prevalence.

**Conclusions**

At the time of writing, no epidemic strains of *Clostridium difficile* have been detected in Australia (Elliott B, Chang BJ, Riley TV; unpublished observations), and we have found a low prevalence (1–2%) of strains resistant to the newer fluoroquinolones in both eastern and western Australia. These strains were collected over the last two–three years. Similar low resistance levels have been reported previously for a selection of strains collected during the 1990s in Sydney [9], suggesting that there have been no significant changes in susceptibility patterns during this period.

It remains to be seen whether this will continue. What is required is continued monitoring of the situation, with periodic regular testing of isolates for changes in susceptibility patterns, as well as molecular typing to conclusively establish the presence of strains with epidemic potential. This will require diagnostic laboratories in Australia to make significant changes in their methods, as many have moved away from culturing for *C. difficile* and are using EIA kits in order to save money. In the interests of public health, there is a case for spending comparatively small sums of money now, on surveillance and preparedness, rather than later (as has happened overseas) when patients and healthcare systems have suffered.

**References**