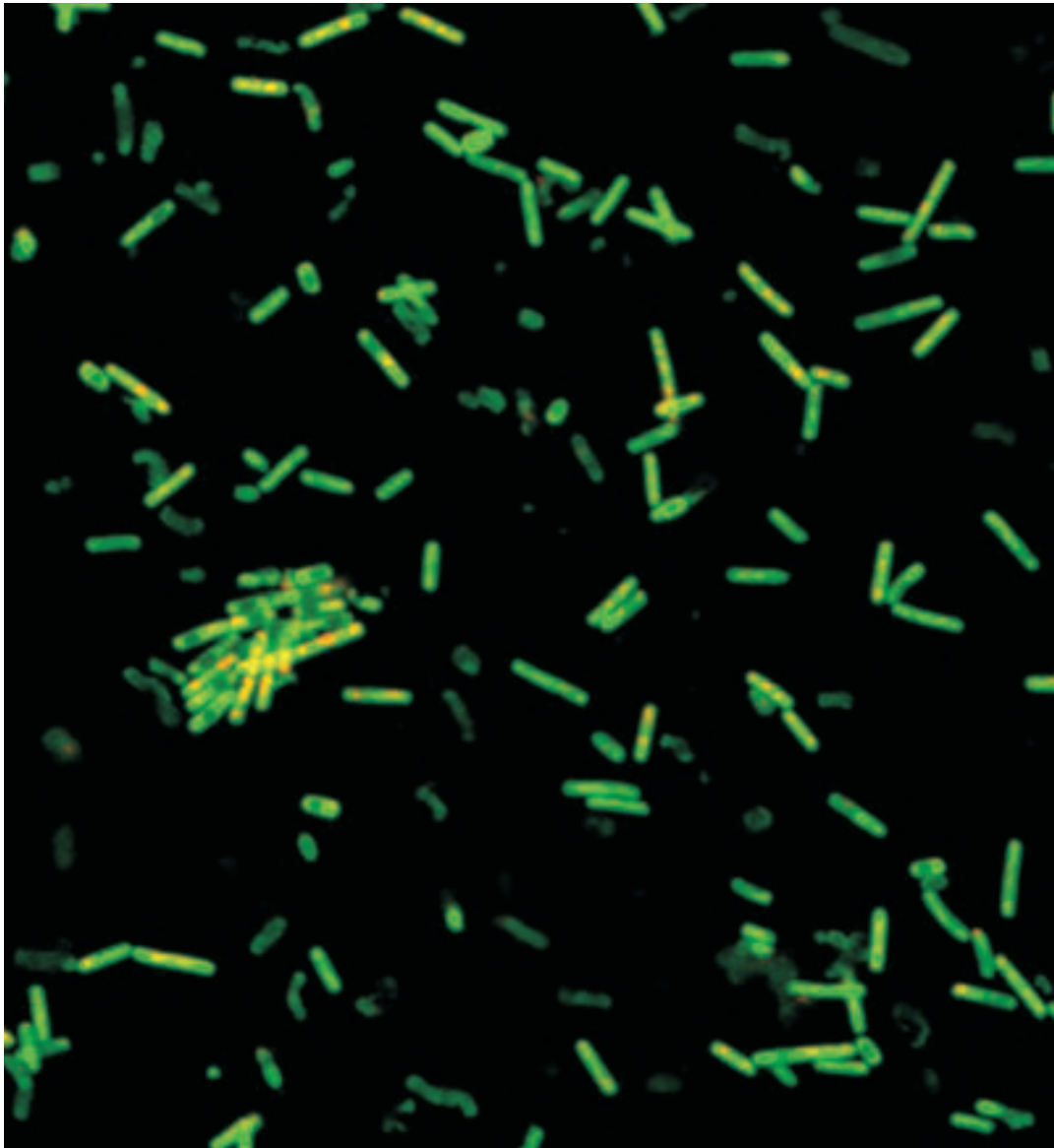


# *Clostridium difficile*: Return of the old enemy

Laura Wheeldon gives us an overview of another hospital-acquired pathogen



**Fig. 1.** *C. difficile* cells fluorescently stained with LIVE/DEAD® BacLight stain and observed under a confocal microscope

*Clostridium difficile* is an anaerobic, spore-forming, Gram-positive bacillus (see figure 1). It was first described in 1935, as a component of the faecal flora in healthy babies and was not recognised as a cause of toxin-associated disease, which ranges from diarrhoea to pseudomembranous colitis, until 1978. *C. difficile* has

now been identified as the leading cause of hospital acquired infectious diarrhoea in adults, with 43,672 reports in the UK in 2004 (Anon, 2005).

## Acquisition of disease and risk factors

*C. difficile* has been cultured from all over hospital wards and acquisition of the

organism occurs primarily in the hospital setting. Organisms have been recovered from beds, toilets, floors, mops and furniture and objects have been found to be contaminated where infected patients were not known to have visited. *C. difficile* has also been isolated from the stools and hands of hospital staff that were asymptomatic for the disease (Fekety *et al.*, 1981). Spores have been found to survive up to 56 days, in temperatures of 4°C and -20°C (Freeman and Wilcox, 2003) and are fairly resistant to many common cleaning agents. Patients most at risk of developing infection are those which have undergone treatment that may impair or disrupt the microflora of the intestine, for example; antibiotics, surgery, immunosuppressive therapy and antacids. Other risk factors include old age, multiple and severe underlying diseases and prolonged hospital stay. Figure 2 shows how risk factors that cause disruption of the flora can enable *C. difficile* to colonise the gut and possibly cause disease.

## Pathogenesis of disease

The pathogenesis of disease can be considered in six stages; entry, adhesion, multiplication, avoidance of host defences, damage to the host and release (Poxton, 2005).

**Entry.** *C. difficile* almost always comes from an exogenous source as it is not detected in the majority of patients and a single strain is often responsible for an outbreak. It is transmitted by

the faecal-oral route, by the ingestion of spores.

**Adherence.** Many adhesins have been described for *C. difficile*, including surface layer proteins (SLPs) and flagella. SLPs are highly immunogenic and patients produce antibody directed against SLPs. It is thought that the flagella may aid in the penetration of the mucus barrier.

**Multiplication.** In order to cause disease there must be sufficient numbers of the organism to produce toxin. As the use of broad spectrum antibiotics eliminate much of the normal flora of the intestine, *C. difficile* is able to multiply without much interference by competing organisms. Some studies have also shown that more toxin is produced by *C. difficile* when it is exposed to sub-lethal concentrations of antibiotic (Drummond *et al.*, 2003).

**Evasion.** It is thought that *C. difficile* uses its wide array of SLPs to evade the immune response of the host along with degradative enzymes and capsules, however none of these have been proven to be involved in evasion.

**Damage to the Host.** *C. difficile* is thought to produce at least two toxins. The two major toxins; toxin A and toxin B are similar in action, causing cell death by disrupting the actin cytoskeleton, after being endocytosed by the host cell. The toxins also induce host inflammatory responses and pseudomembrane formation, (figure 3).

After entrance to the cytosol via passage through an intracellular compartment, the toxins act on the actin cytoskeleton. They cause cell rounding, as cell processes retract due to the disassembly of filamentous F-actin and an increase in G-actin. Before cell rounding occurs, the toxins act enzymatically to modify Rho proteins which regulate

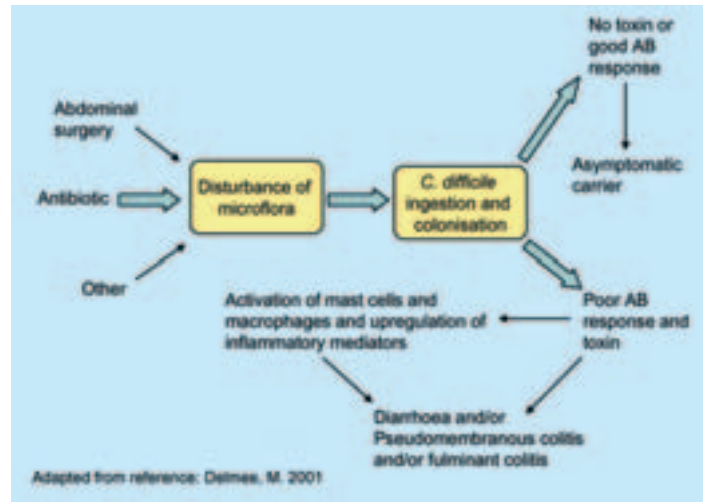


Fig. 2. Adapted from reference: Delmee, M. 2001

fiber assembly and actin polymerization. The loss of functional Rho proteins and the breakdown of actin filaments causes a disruption of the barrier function, by opening the tight junctions between intestinal epithelial cells. This increases permeability in the intestine and causes watery stools, which is a characteristic feature of *C. difficile* antibiotic-associated diarrhoea (Dillon *et al.*, 1995).

Although both toxins have similar structures and mechanisms of action, they differ largely in their potencies, with toxin B approximately 1000 times more potent than toxin A.

Both toxins induce apoptosis of enterocytes and activate the immune system by stimulating the release of TNF $\alpha$  and activating macrophages and monocytes to release IL-8, which causes migration of neutrophils to the site of mucosal inflammation.

Histological changes that may follow include diffuse cell death and ulceration (colitis) and development of a pseudomembrane consisting of mucin, fibrin, leukocytes and cell debris (figure 4).

Some strains produce an additional toxin, a binary toxin, termed CDT. It is thought that this toxin could be an additional virulence factor as it has been shown to

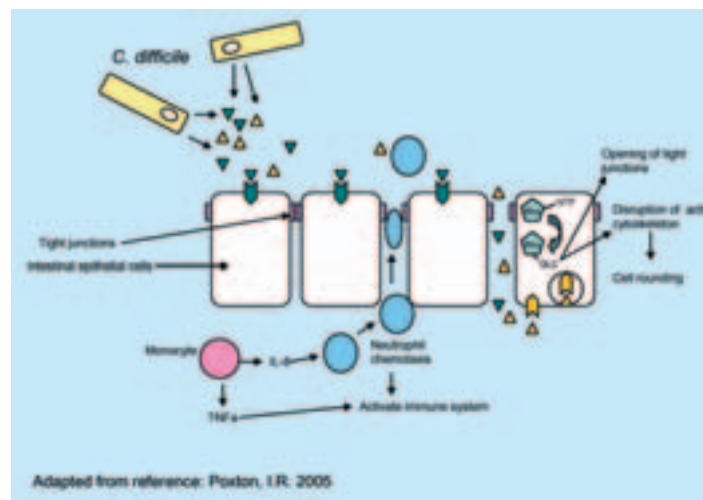


Fig. 3. Adapted from reference: Poxton, I.R. 2005

have cytopathic effects on cell lines that are similar to the other toxins.

**Release.** Release of spores is easily accomplished as *C. difficile* causes diarrhoea, which is often explosive. The spores that *C. difficile* produce act as a reservoir of infection and make it possible for the organism to survive in aerobic conditions. They are extremely hardy and resistant, making them hard to eradicate. Spores are able to survive passage through the highly acidic environment of the stomach and eventually enter the colon, where they may germinate into *C. difficile* bacterial cells.

Contamination of the environment is a major factor in the spread of the organism and it has been estimated that infected patients excrete over 100 *C. difficile* per gram of faeces (Wilcox, 2003).

### Clinical Manifestations of disease

*C. difficile* causes antibiotic associated diarrhoea (CDAD) and colitis; the severity of which ranges from asymptomatic carriage, mild to moderate diarrhoea and pseudomembranous colitis. The mild form of the disease is associated with lower abdominal cramps, but no systemic symptoms. Sparse or diffuse colitis may be evident from endoscopy. A moderate form of the disease is characterized by abdominal pain, profuse diarrhoea and occasionally a small amount of colonic bleeding.

Other symptoms include fever, malaise, nausea and anorexia. There may also be evidence of patchy or diffuse colitis and fecal leukocytes. Approximately 1 - 3% patients develop fulminant colitis, along with toxic megacolon and perforation. In a minority of patients, a reactive form of arthritis may develop, one to four weeks after developing colitis.

## Laboratory Diagnosis

Laboratory diagnosis of *C. difficile* in the stools of a patient is based on two kinds of tests; toxin detection and faecal culture. Unfortunately laboratories differ throughout the UK in that some only use a single toxin detection test or a single immunoassay, whereas others use both toxin detection and culture. Samples are cultured on selective CCFA (cycloserine cefoxitin fructose agar) plates. Sodium taurocholate may also be added to the agar to enhance germination of spores. Culture is a very sensitive method, however it lacks specificity due to the possibility of isolation of non-toxicogenic strains. Toxin testing is more specific and involves inoculating a cell culture (usually HeLa cells) with filtrate of a stool suspension and then observing any cytopathic effects. Enzyme immunoassays are also used widely as they produce results very rapidly; however they have slightly lower sensitivity and specificity.

## Prevention and Treatment

The most important and effective measure to reduce spread of *C. difficile* is a strict cleaning regime of the clinical environment. There is conflicting evidence on best the choice of cleaning agent, but most agree that soap and water is the most effective (Anon, 1994). There should also be strict control over the antibiotics that precipitate disease more readily than others (Schroeder, 2005).

Treatment of *C. difficile* has not advanced much over the past decade; however, some promising new options are currently being explored. The need to find new, alternative treatments for CDAD has arisen from the high relapse rate when initial, conventional therapy is

discontinued. Relapse usually occurs in around 5% - 24% of patients between 1-3 weeks after termination of initial treatment (Buckley, 1996, Pepin *et al.*, 2005). Most relapses are due to infection with new strains of *C. difficile*. The elderly and those who have recently undergone abdominal surgery are more likely to suffer relapse (Young *et al.*, 1986).

The initial treatment of CDAD is termination of the inciting antibiotic whenever



**Fig. 4.** Reprinted with permission: *Biomedical Scientist* 2005; 49: 1034-40. © Institute of Biomedical Science. I Poxton

possible or the switch to a narrow spectrum antibiotic. In cases of mild diarrhoea this may be adequate to resolve symptoms; however, most require further treatment with one of two antibiotics; metronidazole or vancomycin. Hydration and electrolyte replacement therapy may also be required in conjunction with antibiotics in severe cases and in the young and elderly.

Metronidazole and vancomycin are the main antibiotics used to treat CDAD. Metronidazole is usually the treatment of choice as it is as effective, but not as costly as vancomycin and vancomycin should really be reserved for treatment of MRSA.

Alternative treatments options are very important in pursuing as most antibiotics only target vegetative cells. Other possible therapy options for treatment of CDAD include: probiotics, adsorbents, immune products, faecal enemas and bowel irrigation and a series of new compounds. Identifying which strain of *C. difficile* is causing disease may also aid in deciding which treatment will be most effective (McFarland, 2005).

Probiotics are living organisms, which have specific therapeutic properties and inhibit growth of pathogenic bacteria. The normal healthy gut has colonization resistance, which prevents pathogenic bacteria from attaching to the gut and causing disease. When the balance is disrupted due to antibiotics or other disruptive factors the luminal wall is susceptible to infection by pathogenic bacteria. Probiotics help to re-colonise the gut with non-pathogenic bacteria, thus preventing the attachment of harmful bacteria.

*C. difficile* has been a major cause of nosocomial infection for many years, but until now it had not gained the

attention of the media. With the organism now being hailed as 'the new superbug' will awareness now increase and will more be done to decrease infection rates in our hospitals?

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