**Clostridium difficile** infection of the gut

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**Introduction**

Many species of clostridia are capable of causing disease of the gut, the most common of which in hospitals is that due to *Clostridium difficile*. *C difficile* is now recognised as the cause of pseudomembranous colitis (PMC) and many cases of antibiotic associated diarrhoea, although some cases of extraintestinal *C difficile* infection have also been reported. This pathogen is presently of major concern in many hospitals where it is a frequent cause of infection, particularly in the elderly.

PMC was first described as a postoperative complication at the end of the 19th Century and is now well defined histopathologically. However, the causative agent was not known, and it was not until the mid-1970s that its association with antimicrobial therapy was identified.

In the 1940s it was initially thought that PMC was a result of postoperative shock, until the introduction and widespread use of broad spectrum antimicrobial agents, including streptomycin, penicillin and chloramphenicol, indicated a connection between antibiotic use and isolation of antibiotic resistant staphylococci. *Staphylococcus aureus* was implicated as the causative agent. This, however, was not the consensus of opinion. Dawson-Edwards and Morrissey could only find evidence of *S aureus* in one of 35 cases of enterocolitis following gastrointestinal surgery. Furthermore, the association with the use of clindamycin, a highly active antistaphylococcal agent, and cases of PMC was striking. These observations stimulated an intense period of research.

In work carried out in the USA, it was shown that hamsters treated with antibiotics could be cross-infected by intracæcal injection of the caecal contents from diseased animals. Furthermore, the cytopathic effect of caecal filtrates from diseased animals could be neutralised by *C sordellii* antitoxin. This gave rise to the assumption that it was this organism that was responsible for the disease. However, it was not found to be present in the caecal contents of the diseased animals, whereas *C difficile* was. It was soon shown that *C difficile* produces cytopathic toxins that are neutralised by *C sordellii* antitoxin. It is now known that *C sordellii* produces two toxins, haemorrhagic toxin (HT) and lethal toxin (LT) which are very similar to *C difficile* toxins A and B, respectively. In both cases the N-termini of the toxins have a high degree of similarity and each pair of toxins is immunogenically related.

The identification of *C difficile* as the aetiological agent of PMC in humans soon followed the identification of its casual role in experimental animal disease.

**Pathology and pathogenesis**

*C difficile* infection can range from mild diarrhoea to PMC. The symptoms of the disease typically develop after approximately five to 10 days, although this can be delayed for up to two to 10 weeks after discontinuation of antimicrobial therapy. The commonest symptom is a sudden onset of mild diarrhoea, which is foul smelling and contains mucus. Patients with antibiotic associated colitis have profuse diarrhoea, abdominal pain and distension, accompanied by nausea, fever and dehydration.

Gastrointestinal examination of patients with PMC reveals a characteristic picture. At the onset of the disease, 1–2 mm white/yellow plaques appear on the mucosa, which increase in size and coalesce to form larger plaques as the disease progresses. A closer examination reveals typical mushroom-like pseudomembranes erupting from the colonic mucosa, which are comprised of fibrin, mucus, leucocytes, and cellular debris. However, this inflammation is not necessarily confluent in the mucosa and even a single biopsy specimen may show patchy inflammation.

It is accepted that the association between *C difficile* infection and antibiotic therapy is dependant on the disruption of the normal gut microbiota by antimicrobials before the organism can establish and cause disease. This barrier effect to infection afforded by the normal microbiota is commonly referred to as colonisation resistance. It is often the nature of the antibiotic that is important in determining whether a patient will develop a *C difficile* infection, the cephalosporins and penicillins being particularly implicated. In a recent study in Wales, in which 31 of 232 hospital admissions studied tested positive for *C difficile* toxin A, the antibiotics most commonly
associated with these positive cases were those that exhibited a broader spectrum of activity.20

Not everyone seems to be equally susceptible to C difficile infection, the elderly being at the greatest risk.21 It is possible that this higher incidence of infection is due to their poorer colonisation resistance to infection.22 It remains unclear as yet why neonates, who are in the process of producing a complex gut bacterial ecosystem, remain unaffected, even though C difficile and its toxins are frequently present in their faeces.23 24

There are many factors produced by the organism which are thought to contribute to its pathogenicity, including toxin production, protease secretion, presence of fimbriae, and capsule production.25 26 “The most important and widely studied of these is toxin production.

Role and molecular aspects of C difficile toxins

Toxigenic strains of C difficile produce two large protein toxins, toxin A and toxin B, commonly (though erroneously) referred to as the enterotoxin and cytotoxin, respectively, both of which have been cloned and sequenced.27 28 Both are cytotoxic to cells in tissue culture, although toxin B is considerably more cytotoxic than toxin A for most cell lines. Unlike toxin B, toxin A also has the ability to haemagglutinate human red blood cells. Although the toxins share 64% sequence homology, many authors consider that it is toxin A that is active in the intestinal tract and hence responsible for the pathology. However, recent work with human colonic mucosa indicates that toxin B may be approximately 10-fold more active on a molar basis than toxin A in causing damage in humans.29 Unfortunately, before absolute conclusions can be drawn, it would have to be shown that tissue from animals that fails to show damage in response to toxin B alone, remains insensitive to this in the in vitro Ussing chamber model. Nevertheless, the findings are both important and exciting. Studies have shown that toxin B, in the absence of toxin A, has no effect on the gut of experimental animals.

The carboxyl terminal of toxin A consists of 38 repeat units which contain epitopes for the monoclonal antibody PCG-4, used in most diagnostic kits, and includes the binding region for a Galα1-3Galβ1-4GlcnAc carbohydrate receptor, which may be involved in binding of the toxin to cells in the gut.30 It has been suggested that toxin A initiates cell damage which allows toxin B access to the cells beneath the mucosa where it can exhibit its cytopathic effect, resulting in disruption of the cell cytoskeleton.31 The mechanism of action of these toxins is not yet fully understood. It has recently been shown that they both act on the low molecular mass GTP binding proteins, Rho, known to be involved in regulation of the microfilament cytoskeleton.32 33 Giry et al33 have shown that RhoA, RhoB and RhoC are substrates for both toxins, resulting in the disruption of F-actin structures within the cell. Furthermore, toxin B has been shown to glucosylate RhoA specifically, affecting its function.34

Both toxin genes are chromosomal and are located on a 19.6 kilobase toxigenic element, which is comprised of five open reading frames (ORFs), three small ORFs and the toxin A and B genes.35 The function, if any, of the three small ORFs is unknown. This region has recently been shown to be common only to toxigenic strains. In non-toxigenic strains this 19.6 kilobase region of the genome is completely absent and in its stead there is a stretch of 127 base pairs of non-coding DNA, although the flanking regions in this part of the genomic DNA are identical in both toxigenic and non-toxigenic species.36 This is indicative of a transpositional event in the acquisition or deletion of the toxin genes.

As yet, no strain has been found that contains only one of the toxin genes, although there is a report that a toxin B positive/toxin A negative strain had been isolated, which was virulent in a hamster model.37 The toxin B of this strain seemed to be more toxic than that produced by the classically well defined strain VPI 10643. It later emerged that this strain did in fact have the first 2 kilobases of the toxin A N-terminus.38 However, what effect this had on the pathogenicity of the organism is unknown.

Some variation in the size and DNA sequence of the toxin B gene has been found between certain toxigenic C difficile strains.37 38 A correlation between these changes and the cytopathicity of these different toxins has been drawn and has given some insight as to which areas of the toxin may be responsible for the cytopathic effect seen. Eichel-Streiber et al,35 in comparing toxin B produced by two toxigenic strains, which have distinctly different levels of cytotoxicity, have shown that the majority of the changes in the amino acid composition of the two toxins are clustered within the N-terminal third of the proteins, and concluded that it is this domain that carries the toxic determinants.

Diagnosis and treatment

Isolation of C difficile from faecal samples, although important, is not in itself a conclusive diagnostic test for disease,39 as it is possible to harbour the organism asymptptomatically. There are commercially available enzyme linked immunoassay kits40 that rely on the detection of toxin A in faecal samples, based on the PGC-4 monoclonal antibody reaction with part of the repeat sequence of the toxin A molecule. These kits provide a rapid and reliable method of diagnosis and further confirmation may be obtained by application of faecal filtrates to cells in tissue culture and monitoring cytopathic effects.

Treatment of the infection can often be achieved by cessation of the offending antibiotic. Although diarrhoea may cease within 48 hours, care must be taken to ensure replenishment of lost fluids. In cases where specific treatment is indicated vancomycin or metronidazole are the drugs of choice.41
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Relapses of C difficile colitis are not uncommon and can be more difficult to treat. The use of Saccharomyces boulardii as a biotherapeutic agent in conjunction with oral vancomycin has been shown to reduce the failure rate of treatment. Recently, a report has shown that treatment with brewer's yeast (S cerevisiae) alone has enabled four patients, who had suffered relapses even after extensive treatment with vancomycin, to recover completely from a C difficile infection. However, this was an uncontrolled study and further work is necessary to assess the viability of such a therapy, which, if successful, could provide an inexpensive alternative to antibiotic treatment.

Epidemiology and control

It is still unclear whether existing C difficile in the gut or exposure to an external source at the time of susceptibility is responsible for infection, although the weight of evidence is in favour of infection in the vast majority of cases. Hamster models have shown that clindamycin treated animals did not develop C difficile infection if held in a protective environment, and further showed that C difficile may be transmitted from one animal to another. In a clinical environment spread of the organism is thought to be the result of ingestion of spores. These are stable in the environment for several months and contamination of floors, toilets, bedpans, and furniture can arise, especially in areas where patients with C difficile infection have been treated. Patients with existing C difficile infections are thought to be the main source of infection for other patients and although asymptomatic excretion may play some part, there is no conclusive evidence as yet. Reported carriage rates in healthy adults have varied from 0–3% in Europe and up to 15% in Japan, although these differences may only be due to variations in subject selection and methods of isolation of the organism. One study has shown that asymptomatic carriage increased to 48% after the subjects were given antibiotics. In the community it has been suggested that pets may also play a role as a source of C difficile infection. Enteric infection control methods should include isolation of infected patients and wearing of disposable gloves and aprons, strict hand washing, rigorous cleaning procedures, including “deep” cleaning of wards where several cases have occurred, restriction of staff and patient movement, and restriction of antibiotic usage. It is possible to keep groups of affected patients in a single ward, as long as they are “cohort nursed”. After initiation of such methods during a serious outbreak in Manchester, there was a substantial and sustained decrease in the number of new cases. For further information, readers are directed to a recent review of the control and treatment of C difficile infections.

Conclusions

Although a great deal has been learnt about C difficile in a relatively short period of time, including identification of the intracellular target of the toxins, there remains much still to be understood. Why infants seem to be resistant to the action of the toxins, and whether or not there may be rare exceptions to this and subsequent disease development, needs further study. The availability of diagnostic kits for rapid detection of toxin has greatly enhanced the ability of pathology laboratories to identify cases of C difficile infection. However, little thought has accompanied their widespread use regarding the possible over aggressive response in treatment. By and large, the response to a positive laboratory finding is immediate specific treatment with vancomycin or metronidazole, even though it is well established that cessation of the antibiotic, when possible, is often sufficient. The extent to which “unnecessary” treatment contributes to recurrent bouts of infection is unknown, but it is the opinion of the authors that this is undoubtedly so in some cases. The real problem of treating recurrences and relapses is also still unresolved. Unfortunately, the increasing numbers of hospitalised elderly patients, the highest risk patient group for C difficile infection, means that the incidence of this infection will continue to rise, and may eventually rival MRSA as the most common identifiable nosocomial infection.


