

**Witness Report to the *Clostridium Difficile* Public Inquiry*****Clostridium Difficile*: Antibiotic Associated Diarrhoea****George E. Griffin BSc PhD FRCP F.Med Sci****Professor of Infectious Diseases and Medicine****St. George's, University of London**General

Diarrhoea has been a well-known feature of treatment with antibiotics and is described as antibiotic associated diarrhoea (AAD). As the use and types of antibiotic agents dramatically increased from the 1960's AAD was progressively observed as a greater problem and case reports were published in the late 1970's documenting a very severe and occasionally fatal form of the disease, *Pseudomembranous colitis* (PMC). It was then appreciated that AAD ranged from a simple self-limiting condition, which disappeared on cessation of antibiotic treatment, to fulminant PMC. Initially the definitive cause of the diarrhoea was unknown and treatment was purely symptomatic, sometimes in severe case culminating in removal of the colon (colectomy) known to be the affected organ. Definition of the aetiology of the condition was slow and early findings suggested a bacterial origin (*Staphylococcus aureus*) but this was refuted. Since at this time no bacterial cause was apparent, research centered around a viral cause of the colitis and classical virological methods were employed to detect virus in stool. This involved taking fluid from stool, filtering to remove bacteria and putting this onto tissue culture cells in an attempt to grow virus. It became immediately apparent that such tissue culture cells changed shape very quickly becoming rounded up (actinomorphous change). Thus research focused on identification of the toxic substance in stool responsible for such

effects. After some serendipitous experiments using the stool filtrate-tissue culture system, it was discovered that the toxin causing the cells to round up was completely neutralised by antiserum against a mixture of *Clostridial* toxins (see later). Further analysis eventually revealed that the specific toxin was from *Clostridium difficile* and that this microorganism was present in virtually all diarrhoeal stool from AAD affected patients. Since this seminal early finding there has been much research focused on the clinical disease, diagnostic methods, microbiology of *C. difficile* and its toxins, epidemiology of carriage in stool, clinical incidence, management and control. Whilst much has been learned about the organism, the disease it causes and its management, there are still many unanswered questions and considerable research is in progress. However, using existing knowledge, considerable steps have been made to reduce incidence and control the disease. (See General Review<sup>1</sup>)

#### **i) Clinical Disease and Microbiological Diagnosis: Antibiotic Associated Diarrhoea**

The clinical manifestations of AAD range very considerably from simple loose stool to fulminant watery/bloody diarrhoea with abdominal pain, which can be associated with dilation and perforation of the colon requiring major abdominal surgery. The latter fulminant form of the disease is fortunately rare. Diarrhoea may be present within a few days of starting an antibiotic course but may be delayed until after the course of antibiotics is complete. Virtually all antibiotics have been implicated as being associated with AAD but in terms of prevalence the commonly used B lactams (e.g. amoxicillin), quinolones (e.g. ciprofloxacin) and cephalosporins (e.g. cefotaxime) account for most cases. Repeated courses of different antibiotics also predispose to developing the disease.

AAD may affect any age group, except neonates up to the first few months of life (see later). However, the disease is of particular significance in any immobilised bed bound patient, posing a great challenge to nursing care and obvious implications for cross infection, requiring isolation. Severely ill post-operative patients (e.g. multiple trauma) and demented bed bound patients pose the biggest challenges for care and treatment.

The self-limiting form of AAD usually requires no specific treatment in the ambulant patient and will resolve within around a week after cessation of antibiotic treatment. More severely affected patients require careful management of fluid and electrolyte balance and specific antimicrobial medication directed against *C. difficile* (see later). In patients with severe disease, *Pseudomembranous colitis*, very close abdominal observation is essential to detect colonic dilation (clinically and using abdominal radiology) and measurement of markers of progressive inflammation (e.g. C-reactive protein) in plasma gives an indication of progression. After successful treatment and resolution of AAD relapse of clinical disease is common and may affect up to 30% of patients. Relapse is treated in the same symptomatic supportive way and change of antibiotic against *C. difficile* is indicated. Reasons for relapse are not fully understood but are likely to involve several factors such as presence of anatomical defects, for example diverticulae and immune response to toxins. A small portion of cases have a very slow response to treatment and the vulnerable, frail and older population, particularly with co-morbidities, are at high clinical risk. Nutritional support is crucial for such patients and poses specific problems in that diarrhoea may be exacerbated.

## ii) *Clostridium difficile*: The Organism, Diagnostic Tests

### a) General

*Clostridium difficile* is a spore forming anaerobic bacterium. This means that it belongs to a group of organisms (*Clostridia*) well known to cause human diseases by production of toxins, which cause severe tissue damage. The anaerobic classification of the organism relates to the fact that the organism does not require oxygen to survive, unlike some other bacteria and human cells which demand oxygen for survival. Indeed oxygen is toxic to many anaerobic organisms and inhibits their growth. *C. difficile* particularly under adverse conditions makes spores which are inert particles containing intact complete *C. difficile* DNA. Such spores can survive for long periods in the environment and are highly resistant to desiccation and chemicals in the resting state. The organism is widely found in the environment, particularly where symptomatic patients have been present (Society for General Microbiology, [www.sgm.ac.uk](http://www.sgm.ac.uk)). In addition, the organism is carried in the stool of some animals but the significance of such carriage to human disease is currently unknown. As soon as spores reach an appropriate environment (aqueous, anaerobic and containing suitable chemicals for nutritional support) they will germinate to produce new intact bacteria, which replicate and under the appropriate conditions produce and secrete toxins. At least two toxins, A and B, have been implicated in the aetiology of AAD. Toxin A is the toxin which was initially described to cause the cell rounding up in the tissue culture assay system.

Spores or intact organism principally gain access to the body by oral ingestion (Faeco-oral route) and there is now some evidence that aerosol spread may be implicated in spread from symptomatic patients<sup>ii</sup>, raising important considerations for nursing and isolation. After passing through the stomach and small intestine, the local environment in

the human colon is ideal for *C. difficile* to grow and secrete toxins. The toxins act locally on the cells lining the colon (colonocytes) and destroy their integrity and that of the colonic lining. An intense inflammation of the colon (colitis) is thus initiated resulting in diarrhoea. In the most profoundly affected cases, severe inflammation results in significant protein loss from the circulation into the colonic lumen. This protein, classically fibrinogen, forms plaques on the inflamed colonic surface, the pseudomembranes seen in PMC. These pseudomembranes can be seen on examination of the rectum and lower colon by sigmoidoscopy and are pathognomic of PMC.

The question then arises, what is the process that permits the overgrowth of *C. difficile* in patients receiving antibiotic therapy? The human colon, the last part of the intestine, contains faecal material and within this organ there are around  $10^{12}$  (one million million) bacteria per gram of stool. These organisms are mostly anaerobic and in normal individuals pose no clinical threat whatsoever. Bacteria within the colon are in a dynamic equilibrium with each other and this leads to a stable 'micro-flora' which encompasses many different types of microorganisms in a given individual. These organisms are alive and actively dividing. When an individual is given an antibiotic for treatment of an infection (e.g. pneumonia) this antibiotic will act against all bacteria which are susceptible, including the harmless bacteria in the colon. The stable equilibrium of the colonic microflora becomes severely altered and it is this perturbation which gives a niche for *C. difficile* to overgrow, produce toxins and cause AAD and colitis. Thus it is the administration of antibiotics which exposes the individual to overgrowth of *C. difficile* in the colon and potential to cause colitis. It is therefore essential that the prescription and type of antibiotic is carefully considered prior to commencement of such treatment.

The explanation of the cause of AAD presented above is highly simplified and indeed our knowledge of mechanisms is incomplete. For example, factors that are responsible for maintaining normal microflora in the colon are poorly understood and likely relate to dietary intake, colonic structure and immunity. Furthermore, up to 5% of normal, colitis-free adult individuals have *C. difficile* producing toxins in their stool, a carrier state. It is probable that these individuals have inhibitory substances in the colonic lumen or have generated antibodies as part of an immune response to toxins A and B. In addition there is the intriguing observation that of up to 60% normal neonates may carry *C. difficile* and produce toxin in stool, but have no clinical disease. These children could be protected by substances in breast milk or their colonocytes may lack the receptor for the toxins.

#### b) Diagnosis and Ribotyping of *C. difficile*

Diagnosis of AAD is based on clinical suspicion including a history of recent (classically up to six week previously) antibiotic medication. Since there are many infectious causes of diarrhoea it is crucial to make a definitive microbiological diagnosis as this will direct treatment. Diagnosis of *C. difficile* has been achieved in several ways in the past, including identifying the stool toxin in tissue culture and microbiological culture of the organism in stool. However these techniques are laborious and take several days. Consequently, rapid techniques are now available and the latex agglutination technique is used as standard in routine practice. This technique involves using commercially available validated kits which contain latex particles coated within antibodies directed against *C. difficile* toxins, A and B. When *C. difficile* toxins are present in stool the latex particles bind together (agglutination). Such agglutination tests can give positive tests within hours which then can be used to direct appropriate patient management.

/sometimes agglutination tests may be negative and need repeating and algorithms of test to substantiate diagnosis have been devised. In addition some patients, particularly those convalescing from AAD, may have toxin detected in stool but no clinical diarrhoea.

Whilst bacteria such as *C. difficile* are simple life forms they are highly complex biochemically and small variations in their genetic material can alter their biochemical properties and potential to cause disease. Such variations are important in the pathogenicity of *C. difficile*. The first relates to different strains of the organism which can now be identified biochemically by polymerase chain reaction (PCR) ribotyping. In this technique DNA is extracted from the cultured microorganism and specific sections of the DNA (non-translated portions between ribosomal RNA genes) can be expanded biochemically using PCR. These amplified sections of DNA are then analysed and characterized by separating fragments produced by enzymatic digestion of this expanded DNA on a gel. Specific patterns of these DNA fragments are generated and are used to categorise the type of *C. difficile* (ribotyping). Several typing systems have been devised for *C. difficile*<sup>iii</sup> and ribotyping has been chosen for use in the UK<sup>iv</sup>, performed by the *C. difficile* Ribotyping Network for England (CDRN) coordinated by the Health Protection Agency. Northern Ireland was incorporated into CDRN in 2009. This has resulted in valuable information on cross infection, management of outbreaks and determination of epidemiology of infections. At least ten genetically different ribotypes of *C. difficile* have been identified in the UK and epidemiological studies have clearly demonstrated changes in the prevalence of the different ribotypes over time (see later). Furthermore, genetic analysis of the organism has led to the discovery that a hypervirulent strain of *C. difficile* (027/B1/NAP1) has evolved which leads to more serious disease because this organism produces and releases more toxin.

### **iii) Treatment of *Clostridium difficile* infection**

The aim of definitive antimicrobial treatment of AAD is to remove *C. difficile* from the colon, and therefore the source of the toxin, allowing the colon to regain normal function as the inflammatory process is reduced. The consensus view is that metronidazole is the first line of antimicrobial treatment. However the use of this antibiotic is not universally successful, with up to 25% clinical failure rate. Such failure may relate to low level of this antibiotic in the colonic lumen and the discovery of varying degrees of *C. difficile* resistance to this agent. Vancomycin is an antibiotic not absorbed in the small intestine and therefore when given orally reaches the colon in high concentration and is active against *C. difficile*. However there is concern that other organisms in the colonic lumen may develop resistance to vancomycin and themselves become serious pathogens e.g. vancomycin resistant enterococi. This was a principal reason, in the USA particularly, to reserve vancomycin as treatment for relapses or serious cases.

The use of probiotics (e.g. those in yoghurt) has been advocated as a way in which the colonic microflora may be encouraged to reconstitute and assume a normal equilibrium after antibiotic treatment. However definitive proof that this probiotic approach works is lacking. Reconstitution of the normal faecal microflora in patients with AAD using faecal enemas obtained from well individuals has not proved successful. In addition the intravenous use of pooled immunoglobulin (e.g. Sandoglobulin) is controversial and very expensive but has some scientific basis in that antitoxin antibodies may be present. Both of the latter therapies are under further investigation.

In addition, immunologically based therapies are under investigation at the moment and are promising. These include vaccination of at risk individuals with vaccine consisting of

inactivated *C. difficile* toxins. In addition, the use of antibodies directed against *C. difficile* toxins administered by oral, rectal or intravenous routes is currently under active investigation. It is now commercially possible to produce such antibodies in large quantities using recombinant techniques. Both of these techniques offer potentially important ways of preventing AAD (active immunity) and treating disease when it is established (passive immunity). It is interesting to note that the appearance of specific antibodies to Toxins A and B in blood of individuals suffering from severe relapsing disease correlates with cure. Such observations lead to hope that immunological treatment will be clinically useful. However using simple measures of control, deaths in England and Wales from *C. difficile* have halved between 2007 and 2009 (National Statistics Online - Clostridium Difficile)

#### **v) Epidemiology and Control of Infection**

The epidemiology of *C. difficile* infection is changing rapidly<sup>v</sup>. Since the serious nature of the disease and its prevalence have been recognized in the UK, there has been a 35% fall in cases in patients aged two and over (36,097 *C. difficile* infections in 2008/9 from 55,499 in 2007/8<sup>vi, vii</sup>). During this period, there has been a 20% fall in infections with the predominant ribotype (027), however this is compensated by rises in prevalence of other ribotypes. The prevalence of AAD in the older population, particularly in nursing homes is of major significance. The carriage rate of the organism in such populations is thought to be high and is under investigation. The fall in *C. difficile* infections reflects steps taken as a result of greater understanding of the aetiology of infection, rational use of antibiotics, improved cross infection practice and case isolation<sup>viii</sup>. Most hospitals now have guidance on clinical use of antibiotics recommending careful consideration of the clinical need for antibiotic treatment and reduced use particularly of cephalosporins and

quinolones. Furthermore, the use of rigorous hand washing, disposable clothing and isolation of patients with AAD have become routine but pose great logistical problems within institutions. The spores of *C. difficile* are resistant to alcohol hand washes/wipes and hand washing using soap and water is crucial. Since spores of the organism are known to be highly resistant, the recommended cleaning and disinfection procedures for contaminated surfaces is hypochlorite (bleach) and protocols have been produced giving clear advice.

## References

- 
- <sup>i</sup> *Clostridium difficile* — More Difficult Than Ever  
Kelly C. P. and LaMont J. T.  
N Engl J Med (2008) 359:1932-1940
- <sup>ii</sup> The potential for Airborne Dispersal of *Clostridium difficile* from symptomatic patients  
Best E.L., Fawley W. N., Parnell P. *et al*  
Clinical Infectious Disease (2010) 50;1450-1457
- <sup>iii</sup> Comparison of seven techniques for typing international epidemic strains of *Clostridium difficile*  
Killgore G, Thompson A, Johnson S *et al*.  
J Clin Micro (2008) 46;431-437
- <sup>iv</sup> *Clostridium difficile* Ribotyping Network for England and Northern Ireland 2008-09 report pub  
Health Protection Agency
- <sup>v</sup> The Changing Epidemiology of *Clostridium difficile* Infection  
Freeman J., Bauer M.P., Baines S.D. *et al*  
Clinical Microbiology Reviews (2010) 23;529-549
- <sup>vi</sup> Healthcare-associated Infections in England 2008-2009 Report. Pub Health Protection Agency  
([www.hpa.org.uk](http://www.hpa.org.uk))
- <sup>vii</sup> Mandatory *C. difficile* Pub Health Protection Agency  
([www.hpa.org.uk/weebw/HPAweb&HPAwebAutoListName/Page/1191942169773](http://www.hpa.org.uk/weebw/HPAweb&HPAwebAutoListName/Page/1191942169773))
- <sup>viii</sup> Prevention and Medical Management of *Clostridium difficile* infection  
Shannon-Lowe J., Matheson N.J., Cooke *et al*  
BMJ (2010) 340;641-644

**EXPERT WITNESS STATEMENT TO THE PUBLIC INQUIRY INTO THE  
OUTBREAK OF CLOSTRIDIUM DIFICILE IN THE NORTHERN TRUST  
HOSPITALS**

EXPERT WITNESS NAME: Prof. George Griffin

I hereby attach a report dated \_\_\_\_\_ which forms my written  
statement of evidence to this Inquiry.

I declare that this statement is true and accurate to the best of my knowledge and  
belief.

Signed: \_\_\_\_\_

Date: \_\_\_\_\_

*Please return your report.*