Article:

Evidence for antibiotic induced *Clostridium perfringens* diarrhoea

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Abstract

***Clostridium difficile* is a well documented cause of antibiotic associated diarrhoea in hospitalised patients, but may account for only approximately 20% of all cases. This leader reviews the current knowledge and understanding of the pathogenesis, epidemiology, and diagnosis of non-food borne *Clostridium perfringens* diarrhoea. Although enterotoxigenic *C perfringens* has been implicated in some *C difficile* negative cases of antibiotic associated diarrhoea, *C perfringens* enterotoxin detection methods are not part of the routine laboratory investigation of such cases. Testing for *C perfringens* enterotoxin in faecal samples from patients with antibiotic associated diarrhoea and sporadic diarrhoea on a routine basis would have considerable resource implications. Therefore, criteria for initiating investigations and optimum laboratory tests need to be established. In addition, establishing the true burden of *C perfringens* antibiotic associated diarrhoea is important before optimum control and treatment measures can be defined.**
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Clostridium difficile is the most commonly identified pathogen in hospital acquired infective diarrhoea. However, for most cases (in some series up to 80%) the organism responsible is undiagnosed. *Staphylococcus aureus* and *Clostridium perfringens* are the most frequently cited alternative causes of antibiotic associated diarrhoea.[1] *Clostridium perfringens* type A is an important cause of bacterial food poisoning world wide. There is mounting evidence to suggest that enterotoxigenic *C perfringens* strains can play a role in the aetiology of diarrhoeal disease distinct from food poisoning, including antibiotic associated diarrhoea and sporadic diarrhoea in humans.[2–9] *Clostridium perfringens* forms part of the normal human gut flora in small numbers—up to 10^3 colony forming units (cfu)/g. Ingestion of large numbers of vegetative cells ($\geq 10^8$ enterotoxin producing organisms) of *C perfringens* leads to diarrhoeal illness, as in cases of food borne disease. The organisms multiply in the small intestine and sporulate, releasing *C perfringens* enterotoxin (CPEnt), which is responsible for the classic symptoms of food poisoning. Faecal counts of $\geq 10^6$ *C perfringens* spores are seen in symptomatic cases, although similar counts can be obtained in patients without symptoms.

Experimental diarrhoea has been produced in human volunteers after oral administration of CPEnt.[10] More recently, CPEnt has been implicated in the pathogenesis of antibiotic associated diarrhoea and sporadic diarrhoea. The aim of this article is to summarise the available evidence for *C perfringens* as a cause of antibiotic associated diarrhoea and provide an overview of the laboratory methods for diagnosis of *C perfringens* diarrhoeal diseases.

Evidence linking *C perfringens* to non-food borne diarrhoeal disease

In 1984, Borriello *et al* demonstrated that some *C difficile* toxin negative cases of antibiotic associated diarrhoea were associated with enterotoxigenic strains of *C perfringens*.[2] In their study, faecal specimens from patients with antibiotic associated diarrhoea were examined. Faecal specimens were screened by tissue culture assay for CPEnt, and the presence of enterotoxin was further confirmed by an in house enzyme linked immunosorbent assay (ELISA). All the cases of diarrhoea had high faecal counts ($> 10^6$ cfu/g) of *C perfringens.* *Clostridium perfringens* enterotoxin was detected in the stools of 11 patients with diarrhoea. The severity, duration, and sporadic nature of the disease was not characteristic of *C perfringens* food poisoning, and most (n = 10) of the patients developed diarrhoea after antibiotic treatment. Implicated antibiotics included penicillins, cephalosporins, trimethoprim, and cotrimoxazole. Furthermore, CPEnt was not detected in the stools of patients with inflammatory bowel disease (n = 29) and infective diarrhoea (n = 12), or in patients with normal stools (n = 16). These findings led the authors to conclude that enterotoxigenic *C perfringens* was a possible cause of antibiotic associated diarrhoea. Symptoms associated with non-food borne *C perfringens* diarrhoeal disease tend to be protracted and more severe than those of food poisoning.[11] Common symptoms include abdominal pain and diarrhoea, which are often accompanied by blood and mucous in the faeces. Borriello and colleagues subsequently confirmed their earlier findings by extending the series to 50 patients.[3]

Samuel *et al* studied the incidence of diarrhoea associated with CPEnt by screening diarrhoeal samples received from both general practice and hospital patients.[4] In addition to screening for common enteric pathogens, samples were also tested for the presence of *C perfringens* and its enterotoxin. Of the 721 diarrhoeal specimens examined, 25 were shown to contain CPEnt accompanied by high clostridial counts ($> 10^3$ cfu/g). The control

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group samples, consisting of 120 normal stool specimens, were negative for CPEnt and had low faecal counts of *C perfringens* ($\leqslant 10^3$ cfu/g). The study demonstrated a clear association of *C perfringens* diarrhoea with elderly hospitalised patients, with 22 of 25 of the cases being hospital inpatients. Most (n = 18) of the patients had received antibiotics before the onset of diarrhoea. The antibiotics associated with the cases were penicillins, cephalosporins, augmentin, erythromycin, trimethoprim, and nitrofurantoin. The clinical symptoms developed on different days over a period of a few weeks and were not typical of food poisoning. A more recent study was designed to investigate the incidence of *C perfringens* as a cause of antibiotic associated diarrhoea.[5] Hospitalised patients were assigned to one of the four groups on the basis of antibiotic usage and diarrhoeal disease. Patients with infective diarrhoea caused by other enteric pathogens were excluded from the study. The control group (n = 181) comprised patients who had or had not received antibiotics but had no diarrhoea. There was no predominant age group or sex. The stool samples were examined for the presence of *C difficile* and *C perfringens* toxins. CPEnt was detected in the faeces of 15 patients with antibiotic associated diarrhoea, but in none of the control groups. A further four patients with diarrhoea had detectable CPEnt, although there was no association with antibiotic treatment. The possibility of cross infection was suggested on the basis of geographical clustering, but isolates were not available for strain typing. However, an epidemiological investigation carried out elsewhere during an outbreak of *C perfringens* diarrhoea showed that infecting serotypes were present in the hospital environment and on the hands of infected patients.[6] *Clostridium perfringens* serotypes recovered from symptomatic patients were present in 59% of related environmental samples. Only 9% of samples from areas not associated with symptomatic patients were similarly positive. In a study from Japan, *C perfringens* isolates recovered from the faeces of elderly hospitalised patients with sporadic diarrhoea were DNA fingerprinted using pulsed field gel electrophoresis (PFGE).[7] Of the 60 *C perfringens* isolates examined, 38 shared the same DNA PFGE pattern. Epidemiological and experimental data suggested that the diarrhoea was not related to a food borne outbreak, but was caused by a nosocomial spread of the bacterium.

Sporadic cases of diarrhoea in the community have also been caused by *C perfringens,* particularly in the elderly population.[8 9] In a study by Mpamugo *et al*, faecal specimens from isolated sporadic cases of diarrhoea were examined using a reverse passive latex agglutination (RPLA) kit (see below) for enterotoxin detection, and positive results were confirmed by an in house ELISA.[9] Enterotoxin was detected by ELISA in 43 of 45 specimens that yielded (in the RPLA kit) a difference of two wells or more between sensitised and control latex, and in 22 of 56 specimens with a one well difference. Hence, enterotoxin was detected in

65 of the 212 faecal specimens examined. Most of the enterotoxin positive cases were not associated with antibiotic treatment. In some of the cases, a single point source of food borne organisms could not be excluded. In addition, a higher proportion (79%) of the enterotoxin positive group than the enterotoxin negative group was resident in the community. The importance of these findings remains uncertain, but the surprisingly high prevalence of enterotoxin positive cases raises questions about the specificity of the detection methods used. In a recent large study of infectious intestinal disease in the community in England, CPEnt was found at a much lower prevalence than in the study by Mpamugo *et al*, but was more frequently identified in general practitioner patients (4%) than in the population cohort (1.2%).[12]

Thus, evidence supporting a causal role for *C perfringens* in non-food borne diarrhoeal disease includes the presence of enterotoxin in the faeces of patients with antibiotic associated diarrhoea or sporadic diarrhoea, as opposed to asymptomatic individuals or individuals suffering from infectious intestinal disease caused by other pathogens.[13 14] It might be argued that patients identified as suffering from *C perfringens* antibiotic associated diarrhoea were in fact cases of food poisoning. Although it is difficult to eliminate the possibility of food poisoning completely, the clinical symptoms described above were not consistent with those of food poisoning. There were no case clusters in relation to time to suggest a common source food borne outbreak. In a recent study, Collie *et al* used restriction fragment length polymorphism (RFLP) and PFGE to genotype 43 enterotoxigenic *C perfringens* isolates.[14] Results of their study showed that the enterotoxin gene (cpe) is extrachromosomal in *C perfringens* isolates associated with non-food borne diseases in contrast to the chromosomal enterotoxin gene present in food poisoning isolates.[14] The importance of this observation is unclear, but it does strengthen the possibility that *C perfringens* antibiotic associated diarrhoea is a distinct entity. Furthermore, these findings may be useful for identifying reservoirs of genotypically distinct subpopulations of enterotoxigenic *C perfringens* isolates, which might help in instituting appropriate preventive measures.

Pathogenesis
There is a paucity of data relating to the pathogenesis of *C perfringens* antibiotic associated diarrhoea. Use of antibiotics in elderly hospitalised patients is considered the major risk factor for acquisition of the disease. It is not known whether antibiotic exposure primarily permits the proliferation of small numbers of resident *C perfringens* strains or allows acquisition of enterotoxigenic *C perfringens* strains—for example, as a result of impaired colonisation resistance. The symptoms are mediated by the production of CPEnt, which is a 35 kDa polypeptide.[14 15] The cpe gene has been cloned, sequenced, and expressed in *Escherischia coli*.[16] Further studies are required to assess the extent of genomic diversity among the food

borne and non-food borne diarrhoeal disease isolates. These studies may also help to explain the differences in virulence between the genotypically distinct subpopulations of enterotoxigenic *C perfringens* isolates. It is widely believed that sporulation is essential for CPEnt production. However, Czeczulin *et al* reported that sporulation is not essential for CPEnt production, although it does lead to an increased yield of CPEnt.[16] At present, regulation of CPEnt expression at the molecular level is not well understood. Studies using gene probe assays have shown that cpe is present in only 6% of global *C perfringens* isolates, whereas 60% of the isolates associated with food poisoning were found to be cpe positive by hybridisation.[17 18] These findings were surprising considering the role of CPEnt in the pathogenesis of *C perfringens* food poisoning. Further studies identified sequence differences in cpe regions that had been used to design some of the DNA probes.[16] These differences may well have interfered with probe hybridisation, resulting in false negative conclusions about cpe positive isolates. It is also possible that some of the isolates tested may have been non-enterotoxigenic normal flora *C perfringens* isolates because only single colonies were selected for testing.[19] More recently, use of gene probe assays based on the cpe sequencing work of Czeczulin and colleagues[16] found that only a small proportion of *C perfringens* isolates carried the cpe gene.[20] Of 454 fresh *C perfringens* isolates from a variety of sources (animal and environmental), only 16 (3.5%) were polymerase chain reaction (PCR) positive for cpe.

Diagnosis

The optimum laboratory investigations for diagnosis of *C perfringens* antibiotic associated diarrhoea have not been established, and the absence of a "gold standard" test hinders further progress. The tests that have been used by previous investigators involve the direct detection of faecal enterotoxin, quantitative stool cultures for *C perfringens*, and absence of other enteric pathogens in stool specimens. Culture of *C perfringens* alone does not prove the diagnosis, particularly in light of the high numbers of asymptomatic carriers in the institutional setting.[21 22] However, stool cultures provide the isolates from which useful data can be obtained (see below).

Clostridium perfringens can be found in small numbers (up to 10^3 cfu/g) in the faeces of healthy individuals.[4] However, there have been reports of high faecal counts of *C perfringens* ($> 10^7$ cfu/g) in specimens obtained from healthy geriatric patients.[21 22] In a study carried out by Yamagishi *et al*,[21] persistently high numbers of *C perfringens* were found in the faeces of Japanese geriatric patients (five of 30). Stringer *et al* studied faecal carriage of *C perfringens* in younger and elderly long stay hospital patients and found similar results in five of 21 elderly patients (counts $> 10^7$ cfu/g).[22] However, the younger long stay patients in the same hospital had faecal counts in the range of 10^3 to 10^4 cfu/g. These findings are in contrast to the findings reported by Samuel *et al*.[4] None of the control

group samples (n = 120) in their study, including those from healthy elderly patients, had faecal counts of *C perfringens* $\geq 10^3$ cfu/g. These conflicting results may be the result of the low numbers of patients recruited in the studies conducted by Yamagishi and colleagues[21] and Stringer *et al*.[22] Therefore, it is essential to detect enterotoxin in faecal samples when attempting to confirm *C perfringens* as the cause of illness.[23] A large number of methods including ELISA, RPLA, and tissue culture assays have been used for the detection of *C perfringens* enterotoxin in faeces, but each has limitations.[24 25] Tissue culture assay, using vero cells, is least sensitive (40 ng enterotoxin/g faeces) and reproducible among toxin detection methods.[24] An in house ELISA available at the Public Health Laboratory Service central food hygiene laboratory has been suggested as the gold standard among toxin detection methods on the basis of its sensitivity (5 ng enterotoxin/g), specificity, and reproducibility.[13 24] The RPLA kit (Oxoid, Basingstoke, UK) has been shown to be both sensitive and reproducible, but non-specific interference by faecal matter has been reported. In addition, some authors have reported that this method has relatively poor sensitivity (50–100 ng enterotoxin/g).[25] Recently, a new *C perfringens* enterotoxin ELISA kit (produced by TechLab; distributed by BioConnections, Leeds, UK) has become commercially available. However, data comparing this ELISA with the methods referred to above are not available. In one study,[5] detection of enterotoxin alone using the new ELISA kit was used as the laboratory diagnostic criterion for inpatients with antibiotic associated diarrhoea. A study undertaken at our hospital using the commercial ELISA kit found that 15% of faecal specimens from patients with antibiotic associated diarrhoea were CPEnt positive (NJ Ransome *et al*, Abstracts of 38th Interscience Conference on Antimicrobial Agents and Chemotherapy, 1998, San Diego. Abstract K 126). This figure is very similar to the results of a previous study.[5] Ten percent of isolates from ELISA negative faecal specimens were cpe positive by PCR (NJ Ransome *et al*, Abstracts of 38th Interscience Conference on Antimicrobial Agents and Chemotherapy, 1998, San Diego. Abstract K 126). It is possible that cpe positive *C perfringens* isolates produced enterotoxin in vivo to cause disease, but there were insufficient faecal concentrations of CPEnt to be detected by ELISA, thus generating false negative results. This could be compounded by the collection of samples late after the onset of illness, because there is evidence that maximum excretion of enterotoxin occurs early in the food borne diarrhoeal illness.[13] Further studies are required to confirm that these findings hold true in cases of non-food borne diarrhoeal illness, and to determine the importance of these preliminary findings. In addition 13 of 19 presumptive *C perfringens* isolates from ELISA positive faecal specimens were cpe negative (NJ Ransome *et al*, Abstracts of 38th Interscience Conference on Antimicrobial Agents and Chemotherapy, 1998, San Diego. Abstract K 126). It is possible

that these specimens contained very low numbers of cpe positive organisms and consequently tested isolates were PCR negative. Furthermore, *C perfringens* isolates can lose the enterotoxin gene during subculture.[26]

Gene probe or PCR assays can be used to determine the enterotoxigenicity of *C perfringens* isolates and thereby avoid the problems associated with culture, and subsequent enterotoxin production in vitro, and the inherent limitations of toxin detection methods. In an attempt to overcome these potential problems, direct detection of cpe in primary faecal specimens by PCR was undertaken in our hospital, using a PCR protocol based on that of Kokai-Kun *et al*.[20] However, all tested faecal samples, including the specimens spiked with enterotoxigenic *C perfringens* reference strains, produced negative results, despite universal bacterial DNA primers yielding positive amplification reactions. It is assumed that the high concentration of background bacterial DNA in stool samples may have interfered with amplification. However, elsewhere PCR assays have been used successfully for the direct detection of enterotoxin genes in faeces.[27 28] For example, Saito *et al* successfully used the supernatant of sporulation cultures of faeces as template DNA to detect cpe by PCR.[28] Further work is needed to overcome the problems associated with the extraction and amplification of *C perfringens* DNA from stool samples. Resolution of this problem could render PCR an important tool for understanding more about *C perfringens* diarrhoeal diseases.

Conclusion

It has been 17 years since Borriello *et al* first demonstrated the role of enterotoxigenic *C perfringens* in the aetiology of antibiotic associated diarrhoea or sporadic diarrhoea, but only limited further research has followed. Current knowledge and understanding of the pathogenesis, epidemiology, and diagnosis of *C perfringens* non-food borne diarrhoeal illness is based on the results of a few published studies, and partly on the extrapolation of data obtained from *C perfringens* food poisoning outbreaks. *Clostridium difficile* is a well documented cause of antibiotic associated diarrhoea in hospitalised patients, but may account for only approximately 20% of all cases.[29] Although enterotoxigenic *C perfringens* has been implicated in some of these *C difficile* negative cases of antibiotic associated diarrhoea, CPEnt detection methods are not part of the routine laboratory investigation of such cases. The criteria for initiating investigations and optimum laboratory tests need to be established. In addition, any diagnostic method used should be rapid, easy to perform, and inexpensive. The performance of the commercial ELISA kit needs to be further evaluated against other methods before being adopted by routine diagnostic laboratories. Testing for *C perfringens* enterotoxin in faecal samples from patients with antibiotic associated diarrhoea and sporadic diarrhoea on a routine basis would have considerable resource implications. In addition, establishing the true burden of *C perfringens* antibiotic associated diarrhoea is important before optimum control and treatment measures can be defined.

1 Wilcox MH. Infective antibiotic associated diarrhoea. In: Wilcox MH, ed. *Infection highlights 1999*. Oxford: Health Press, 2000:57–64.
2 Borriello SP, Larson HE, Welch AR, *et al*. Enterotoxigenic Clostridium perfringens: a possible cause of antibiotic-associated diarrhoea. *Lancet* 1984;i:305–7.
3 Larson HE, Borriello SP. Infectious diarrhoea due to Clostridium perfringens. *J Infect Dis* 1988;**157**:390–1.
4 Samuel SC, Hancock P, Leigh DA. An investigation into Clostridium perfringens enterotoxin associated diarrhoea. *J Hosp Infect* 1991;**18**:219–30.
5 Hancock P. Antibiotic associated diarrhoea C. difficile or C. perfringens? *Reviews in Medical Microbiology* 1997;**8**(suppl 1):S66–7.
6 Borriello SP, Barclay FE, Welch AR, *et al*. Epidemiology of diarrhoea caused by enterotoxigenic Clostridium perfringens. *J Med Microbiol* 1985;**20**:363–72.
7 Wada A, Masuda Y, Fukayama, *et al*. Nosocomial diarrhoea in the elderly due to enterotoxigenic Clostridium perfringens. *Microbiol Immunol* 1996;**40**:767–71.
8 Brett MM, Rodhouse JC, Donovan TJ, *et al*. Detection of Clostridium perfringens and its enterotoxin in cases of sporadic diarrhoea. *J Clin Pathol* 1992;**45**:609–11.
9 Mpamugo O, Donovan T, Brett MM. Enterotoxigenic Clostridium perfringens as a cause of sporadic cases of diarrhoea. *J Med Microbiol* 1995;**43**:442–5.
10 Skjelkvale R, Uemura T. Experimental diarrhoea in human volunteers following oral administration of Clostridium perfringens enterotoxin. *J Appl Bacteriol* 1977;**43**:281–6.
11 Borriello SP. Clostridial diseases of the gut. *Clin Infect Dis* 1995;**20**:S242–50.
12 Tompkins DS, Hudson MJ, Smith HR, *et al*. A study of infectious intestinal disease in England: microbiological findings in cases and controls. *Commun Dis Public Health* 1999;**2**:103–13.
13 Bartholomew BA, Stringer MF, Watson GN, *et al*. Development and application of an enzyme linked immunosorbent assay for Clostridium perfringens type A enterotoxin. *J Clin Pathol* 1985;**38**:222–8.
14 Collie RE, McClane BA. Evidence that the enterotoxin gene can be episomal in Clostridium perfringens isolates associated with non-food borne human gastrointestinal diseases. *J Clin Microbiol* 1998;**36**:30–6.
15 McClane BA, Strouse RJ. Rapid detection of Clostridium perfringens type A enterotoxin by enzyme linked immunosorbent assay. *J Clin Microbiol* 1984;**19**:112–15.
16 Czeczulin JR, Hanna PC, McClane BA. Cloning, nucleotide sequencing, and expression of the Clostridium perfringens enterotoxin gene in Escherichia coli. *Infect Immun* 1993;**61**:3429–39.
17 Van Damme-Jongsten M, Wernars MK, Notermans S. Cloning and sequencing of the Clostridium perfringens enterotoxin gene. *Antonie van Leeuwenhoek* 1989;**56**:181–90.
18 Van Damme-Jongsten M, Rodhouse MJ, Gilbert RJ, *et al*. Synthetic DNA probes for detection of enterotoxigenic Clostridium perfringens strains isolated from outbreaks of food poisoning. *J Clin Microbiol* 1990;**28**:131–3.
19 Rood JI, McClane BA, Songer JG, *et al*, eds. *The Clostridia: molecular biology and pathogenesis*. Cambridge: Cambridge Academic Press, 1997.
20 Kokai-Kun JF, Glenn Songer J, Czeczulin JR, *et al*. Comparison of western immunoblots and gene detection assays for identification of potentially enterotoxigenic isolates of Clostridium perfringens. *J Clin Microbiol* 1994;**32**:2533–9.
21 Yamagishi T, Serikawa T, Morita R, *et al*. Persistent high numbers of Clostridium perfringens in the intestines of Japanese aged adults. *Jpn J Microbiol* 1976;**20**:397–403.
22 Stringer MF, Watson GN, Gilbert RJ. Faecal carriage of Clostridium perfringens. *J Hyg (Lond)* 1985;**95**:277–88.
23 Birkhead G, Vogt RL, Heun EM, *et al*. Characterisation of an outbreak of Clostridium perfringens food poisoning by quantitative faecal culture and faecal enterotoxin measurement. *J Clin Microbiol* 1988;**26**:471–4.
24 Berry PR, Rodhouse JC, Hughes S, *et al*. Evaluation of ELISA, RPLA and vero cell assay for detecting Clostridium perfringens enterotoxin in faecal specimens. *J Clin Pathol* 1988;**41**:458–61.
25 Brett MM. Kits for the detection of some bacterial food poisoning toxins: problems, pitfalls and benefits. *J Appl Microbiol* 1998;**84**(suppl):110S–18S.
26 Brynestad S, Synstad B, Granum PE. The Clostridium perfringens enterotoxin gene is on a transposable element in type A human food poisoning strains. *J Clin Microbiol* 1997;**143**:2109–15.
27 Paton AW, Paton JC, Goldwater PN, *et al*. Direct detection of Escherichia coli shiga like toxin genes in primary faecal cultures by polymerase chain reaction. *J Clin Microbiol* 1993;**31**:3063–7.
28 Saito M, Matsumoto M, Funabashi M. Detection of Clostridium perfringens enterotoxin gene by the polymerase chain reaction amplification procedure. *Int J Food Microbiol* 1992;**17**:47–55.
29 Wilcox MH. Treatment of Clostridium difficile infection. *J Antimicrob Chemother* 1998;**41**(suppl C):41–6.