Project title: The epidemiology of community-acquired Clostridium difficile-associated diarrhoea.

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Background:
Over the last 25 years Clostridium difficile has risen from relative obscurity to be one of the most important hospital pathogens. Data from Sir Charles Gairdner Hospital (SCGH), in Perth, Western Australia, is typical of many, but not all, similar hospitals in developed countries (Riley et al. 1991). SCGH is a 600-bed adult university teaching hospital. During the period 1983 to 1992, C. difficile was detected in 917 patients who were being investigated for diarrhoeal illness. Most of these patients were elderly, with 63% aged 60 years or more, and 59% were female. The number of patients infected each year ranged from a low of 49 patients in 1983 to a high of 120 patients in 1990. When rates were calculated, using occupied bed days as the denominator, a similar trend was observed with the incidence increasing from 23/100,000 occupied bed days in 1983 to 56/100,000 occupied bed days in 1990. Two factors may be particularly important in the rise in infection with C. difficile during the 1980s and 1990s. First, increased and inappropriate use of certain broad-spectrum antibiotics may have predisposed more patients to infection with C. difficile. Second, contamination of the hospital environment with C. difficile represents a significant problem. There are important cost implications for healthcare systems because of this increased incidence of C.difficile-associated diarrhoea (CDAD) (Riley et al. 1995).
The ability of antimicrobial agents to induce diarrhoea was recognised long before *C. difficile* was implicated as a causative agent. Colitis was a common complication of ampicillin therapy, while clindamycin-associated colitis reached epidemic proportions in some parts of the USA in the early 1970s and was, in part, responsible for the research interest which eventually identified *C. difficile* as a causative agent (Bartlett 1979). Some papers have indicated that exposure to clindamycin is still a significant risk factor predisposing to *C. difficile* infection (Golledge et al. 1989). However, during the 1980s and 1990s, in terms of actual usage, third generation or extended spectrum cephalosporins were of greater importance. The increase in incidence of CDAD at SCGH between 1983 and 1990 correlated with an increase in the use of third generation cephalosporins (Pearson’s correlation coefficient, 0.90) (Riley et al. 1991). In Sweden, Aronsson et al. (1984) investigated the relative risk of CDAD for different groups of antibiotics and found that cephalosporins were 40 times more likely to be implicated than ureidopenicillins. Using a case-control methodology, Zimmerman (1991) determined an odds ratio for third generation cephalosporins of 3.00 (p = 0.04), after controlling for horizontal transmission by matching on location. Third generation cephalosporins are known to have a greater impact on colonisation resistance than many other antimicrobial agents.

Recently, there has been great concern world-wide following the emergence in Canada (Loo et al. 2005), the USA (McEllistrem et al. 2005), and now Europe (Kuijper et al. 2006) 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, *C. difficile* disease has been more severe, and attributable mortality was >10% in those aged >60 years (Loo et al. 2005). While the elderly have always been at increased risk of CDAD, due primarily to decreased host defences, rates in persons ≥65 years of age have increased dramatically since 2000 (McDonald et al. 2006).

The outbreak strain of *C. difficile* produces an additional toxin, binary toxin (actin-specific ADP-ribosyltransferase, CDT), first reported in 1988 but not considered important until now (Loo et al. 2005; McEllistrem et al. 2005; Barbut et al. 2005). Binary toxin producers make up the majority of strains isolated in the outbreaks overseas. Barbut et al. (2005) showed 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. Although supernatants from A B CDT⁺ strains of *C. difficile* caused fluid accumulation in a rabbit ileal loop after concentration and trypsinisation, challenge of clindamycin-treated hamsters with these strains resulted in colonisation but not diarrhoea or death (Geric et al. 2006).
Toxigenic isolates of *C. difficile* usually produce two toxins, toxin A and toxin B, and these are thought of as the major virulence factors (Riley 2004). A second important feature of this “new” organism is that it produces more toxin A and B than other strains. Production of these toxins in *C. difficile* is encoded by the 8.1 kb *tcdA* and 7.9 kb *tcdB* genes, respectively. These two genes form part of a highly stable 19.6 kb pathogenicity locus (PaLoc) which also includes *tcdC*, *tcdD* and *tcdE*. Toxin A variant strains fail to produce detectable toxin A by enzyme immunoassay (EIA) because of a deletion in the *tcdA* gene. The *tcdC* gene is a putative down regulator of toxin A and B production. The PCR ribotype 027/NAP1 strain has a deletion in the *tcdC* gene resulting in it no longer down regulating and strains produce toxin throughout log phase of growth instead of just stationary phase (Warny et al. 2005). Non-toxigenic strains lack the PaLoc.

The third important feature of these strains is that they are resistant to fluoroquinolone antibiotics, and excessive fluoroquinolone use appears to be a contributing factor in the recent outbreaks (Pepin et al. 2005). *C. difficile* develops resistance to quinolones soon after exposure (Ackermann et al. 2003), while the gut anaerobe flora remains susceptible. Both the newer fluoroquinolones such as gatifloxacin and levofloxacin and, somewhat surprisingly, the older ciprofloxacin have been implicated (Pepin et al. 2005). Ciprofloxacin has always been thought of as a low risk antimicrobial for inciting CDAD (Golledge et al. 1992), 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.

All the above studies have concentrated on CDAD in a hospital setting. Hirschhorn et al. (1994) examined CDAD in the community setting and calculated antibiotic-specific attack rates. These varied from 0 to 2040 cases per 100,000 exposures and were significantly higher for nitrofuratoin, cefuroxime, cephalexin plus dicloxacillin, ampicillin/clavulanate plus cefaclor and ampicillin/clavulanate plus cefuroxime, than for ampicillin or amoxicillin alone.

Antibiotics are not the only agents capable of inciting CDAD. The role of antineoplastic agents in *C. difficile* infection was reviewed by Anand and Glatt (1993). They reported on all 23 cases of CDAD associated with antineoplastic therapy published in the literature. A variety of antineoplastic agents was implicated, most commonly methotrexate. Laboratory evidence is available to support the case for these agents inciting CDAD, however, the mechanism of
pathogenesis is less clear. Chemotherapeutic agents can alter the gut flora in a manner analogous to many antibiotics and this is probably the most important predisposing factor. The biggest problem in trying to ascertain the importance of antineoplastic agents in CDAD is that many patients who develop CDAD have been exposed to both antibiotics and antineoplastic agents.

CDAD in settings other than hospitals has not been investigated extensively although it is likely to be endemic in many nursing homes. Several studies, including that of Hirschhorn et al. (1994), have focused on CDAD in the community or general practice (Riley et al. 1991; Riley et al. 1995). In the first of these, *C. difficile* or its cytotoxin was found in 16 (5.5%) of 288 stool samples from patients with diarrhoeal illness attending their general practitioners and was the most common enteric pathogen detected (Riley et al. 1991). Most patients had only mild to moderate diarrhoea, however, in the majority, the diarrhoea was protracted.

In a later study (Riley et al. 1995), a larger group of 580 specimens was investigated following a campaign to educate general practitioners about CDAD. There were 75 positive samples (10.7%) from 61 patients and *C. difficile* was the second most frequent enteric pathogen following *Campylobacter* spp. Laing et al. (1996) described three cases of community-acquired *C. difficile* diarrhoeae in elderly patients who had neither received antimicrobial agents nor been institutionalised and Kyne et al. (18) described a community outbreak of CDAD. In a recent study in Sweden, 22% of cases of CDAD were defined as community acquired (Noren et al. 2004). The growing importance of community onset CDAD was highlighted recently by a report of severe CDAD in previously healthy persons and peripartum women (CDC, 2005).

These studies highlight the fact that community-acquired CDAD does occur and that these cases may constitute a significant reservoir of infection for other individuals outside the hospital setting. Unfortunately, little is known of the epidemiology of community-acquired *C. difficile* infection. The most obvious risk factor is still antibiotics, although many recent reports suggest CDAD is being seen in the absence of antibiotic exposure. 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 (Dial et al. 2005), although some case-control studies with hospital patients show no association (Pepin et al. 2005; Loo et al. 2005).
Objectives:
1) To describe the epidemiology of community-acquired C. difficile-associated diarrhoea.
2) To identify risk factors for disease including zoonotic aspects of contamination.

Study design:
A descriptive study followed by a case-control study to determine risk factors.

Study subjects:
All individuals with diarrhoea seeking medical help from their GP during a two month period (January to February) 2010 will voluntarily supply data for questionnaire that will be attached to the request form. Approximately one hundred rural GPs using private pathology laboratories will be asked to supply a minimum of five request + questionnaires each month of the study. All questionnaires will be detached from request form at the laboratory upon test-result and positive C. difficile will represent a case and negative samples controls, matched (± 5 years). From this point the questionnaire will be anonymous numbered consecutively when handed to researcher.

Data collection:
Faecal samples submitted to participating private pathology laboratories for investigation will all be cultured for C. difficile. Incoming faecal samples from community are monitored every week. Attached questionnaire is separated and if positive test labelled case and if negative matched as a control. Each question represents a tentative risk factor and is reported individually to data base.

Data analysis:
Data will be coded and entered into a database created in Epi Info and analysed. Statistical univariate match analysis and applied regression models will be used to evaluate risk factors. Descriptive laboratory data will be subjected to sampling for median differences and chi-square when appropriate.

Microbiological analysis:
Isolates of C. difficile will be typed by molecular techniques and antibiotic susceptibility testing undertaken.

Ethical considerations:
Human Research Ethics Committee approval is sought and chairmans descision is met. Data will be treated as confidential with paperwork stored in a locked cabinet with limited access (named researchers only) and the database stored on one password protected computer.

References:


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