Antibiotic-associated diarrhoea: an overlooked aetiology?

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ABSTRACT

Sixty-three faeces samples from hospital in-patients with probable antibiotic-associated diarrhoea (AAD) are investigated. All samples are examined for routine bacterial enteric pathogens, pus cells, red blood cells and parasites. The samples are also screened for *Clostridium difficile* cytotoxin B (CDT), *C. perfringens* enterotoxin and *Candida* spp. (by microscopy and quantitative culture). Faecal samples from two control groups (healthy volunteers and community samples from GP patients) are also screened. A possible pathogen was found in 71% of AAD cases. *Candida* spp. overgrowth was the most common (44.4%), followed by CDT (34.9%) and *Clostridium perfringens* enterotoxin (9.5%). There was good agreement between the significant Gram films and quantitative *Candida* spp. culture (κ=0.683). Clinical information revealed the majority of patients were on multiple antibiotic regimes, receiving 'high risk' antibiotics. Of community samples, 21% were positive for *Clostridium perfringens* enterotoxin, indicating that *C. perfringens* is a problem in the community, not necessarily associated with antibiotic use. The results suggest that quantitative *Candida* spp. culture should be performed on all specimens requesting AAD investigations.

KEY WORDS: Antibiotic-associated diarrhoea.
Candida.
Clostridium difficile.
Clostridium perfringens.

Introduction

Antibiotic-associated diarrhoea (AAD) is defined as three mushy or watery stools per day, which can develop from a few hours to two months after intake of antibiotics. The condition is a major problem in hospitals, causing distress and discomfort to the affected patients. *Clostridium difficile* is often the only organism considered when investigating AAD. However, only 10–20% of all cases of AAD can be attributed to *C. difficile*.[1] At North Manchester General Hospital, 77% of AAD samples tested are negative for *C. difficile* cytotoxin B (CDT).

The most common antimicrobials implicated in AAD are the broad-spectrum antibiotics. Consensus of opinion suggests that clindamycin is most associated with onset of diarrhoea, followed by the second- and third-generation cephalosporins. These are considered the 'high risk' antibiotics.[2,3]

C. perfringens type A occurs at 10^3 colony-forming units (cfu)/g of faeces in the normal flora of the elderly.[4] It has been suggested that *C. perfringens* may be responsible for non-food related entertoxic disease.[5] The diarrhoea caused is not associated with food poisoning and is more severe, lasting much longer.[6] Antibiotic use is thought to be a significant risk factor, as is age. Although not as common as *C. difficile*-related AAD, *C. perfringens* is a significant cause of AAD.[4,7,8] Diagnosis is based on detection of enterotoxin. It is suggested the organism is an exogenous pathogen with the same cross-infection implications as *C. difficile*.[4,9]

Candida spp. are opportunistic pathogens that may induce disease in the immunocompromised. They are present throughout the normal gastrointestinal tract, and AAD caused by overgrowth of this commensal has been described.[10,11] Overgrowth is defined as $>10^5$ cfu *Candida* sp./mL of faeces.[11] The association of Candida with AAD is controversial, but numerous cases of diarrhoea with no other aetiology, coupled with abatement of symptoms following anti-Candida treatment, have been reported.[10–12]

Candida-associated diarrhoea is characterised by frequent, watery stools, without blood or mucus. Commonly, patients suffer abdominal pain and diarrhoea may be prolonged, lasting three months in some cases. Infection is opportunistic, a decrease in normal flora due to antibiotic use giving organisms such as yeasts a competitive advantage.[10–14] Other predisposing factors leading to candidal enteritis include endocrinological abnormalities and steroid therapy.[13]

Several mechanisms have been suggested to explain why *Candida* spp. overgrowth causes diarrhoea. The presence of mycelial elements in Gram-stained smears of faeces from AAD patients has been noted,[12] suggesting an invasion of the intestinal wall. However, leucocytes are not seen in these smears, indicating a non-invasive disease. *C. albicans* inhibits intestinal absorption of sugar and water, stimulating the net secretion of water, sodium and potassium into the lumen, suggesting diarrhoea is secretory in origin.[10,11]

Laboratory diagnosis is based on quantitation of yeast in faeces. Quantification using Gram-stained smears correlates well with quantitative counts undertaken by serial dilution.[11] Laboratory diagnosis is strongly supported by clinical response to nystatin treatment. Most studies agree that while Candida-associated diarrhoea is not a well-established syndrome, it is implicated in the aetiology of AAD.

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This should be considered, especially in certain groups of patients such as the elderly.[10,11]

The aim of the present study is to investigate the aetiology of AAD.

Materials and methods

Faecal samples met the current guidelines for AAD testing at North Manchester General Hospital: the patient was currently on, or had undergone, antibiotic therapy in the past six weeks; the patient was suffering from sudden-onset, watery, foul-smelling diarrhoea; and the frequency of the diarrhoea was over three times/day.

Sixty-three faecal samples were analysed (33 females, 30 males). The age range of these patients was 26 to 96 years (mean age: 71 years). Two control groups were also included. Control group 1 comprised 20 stool samples from 'healthy' individuals (11 females, nine males), not suffering from diarrhoea. These individuals had not taken antibiotics in the previous six weeks. The age range of this group was 30 to 63 years (mean age: 42 years). Control group 2 comprised 23 diarrhoeal stool samples (12 females, 11 males) from patients in the community. Treatment information on these patients was not fully available. The age range of this group was 36 to 84 years (mean age: 63 years).

Each specimen was examined for the presence of *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Escherichia coli* 0157 and parasitic ova and cysts. Samples were assayed for CDT using Vero cell cultures and for *Clostridium perfringens* enterotoxin using the Techlab enzyme immunoassay (EIA) enterotoxin test. (TechLab, Blacksburg, VA).

The presence of yeasts was quantified by examination of a Gram-stained smear of faeces under oil immersion (x100 magnification). Twenty fields were examined and the average number of yeast cells present per high power field was calculated. This was expressed as 0 = none, 1+ = 1–5 cells, 2+ = 6–10 cells, 3+ = 11–15 cells and 4+ = >16 cells. Bacterial flora present in the smear were also quantified and recorded as 1+ = light, 2+ = moderate and 3+ = heavy.[11]

Liquid specimens were cultured quantitatively using a 2-µL loop on Sabouraud dextrose agar (Oxoid). The plates were incubated at 37°C for 24 h. Two hundred colonies are equivalent to 10^5 cfu *Candida* sp./mL faeces.[15] Solid or formed specimens were quantified using a 0.5-g sample emulsified in 4.5 mL saline (0.85%) to give a 1 in 10 dilution of faeces. Serial dilutions were made to give a dilution of 10^{-3}, and 0.5 mL was cultured as above. Plates were incubated as above.

Fifty colonies are equivalent to 10^5 cfu *Candida* sp./g faeces. Counts of 10^5 cfu/mL or greater were considered positive for *Candida* sp. overgrowth.[11] *Candida* sp. present at 10^5 cfu/mL was identified using the Candida API identification system (bioMérieux). Isolates of *Candida* spp. were typed using Southern blotting by hybridisation assay using a repeat sequence probe at Leeds Mycology Unit, Leeds General Infirmary.

Statistical analysis

The relationship between quantitative *Candida* spp. counts and the presence of yeast cells in Gram-stained smears of faeces was analysed by Kappa (κ).

Results

Hospital patients

A positive aetiology was found in 45 (71%) out of 63 samples (Fig. 1). One sample was positive for $>10^5$ cfu/mL *Saccharomyces cerevisiae*, and 10 patients showed positivity for dual aetiologies.

Eighteen samples were negative for all three investigated agents, and were also negative for routine bacterial and parasitic enteric pathogens. Of these 18, one sample was a formed stool. *Pseudomonas* sp. was isolated from two samples, neither of which yielded other bacterial flora. Four samples had faecal leucocytes present on microscopy. Two samples were negative for any bacterial flora.

Yeast cells were seen microscopically in 25 (78.1%) out of the 28 samples that tested positive for *Candida* spp. overgrowth. There was significant agreement between microscopically detected yeast cells and *Candida* spp. overgrowth (κ=0.683).

Control group 1

All samples from control group 1 ('healthy' volunteers) were formed stools. Each individual was negative for routine bacterial/parasitic enteric pathogens, CDT, *Clostridium perfringens* enterotoxin and candidal overgrowth. Microscopy examination of these samples all showed the heavy presence of bacterial flora but no yeast cells.

Control group 2

All the community samples tested were fluid stools or 'mushy' in consistency. Microscopic examination of samples revealed moderate to heavy presence of bacterial flora. *Candida* spp. counts varied. All samples were negative for CDT. Candidal overgrowth was positive in one sample (4.3 %), three samples (13%) were positive for routine bacterial and parasitic enteric pathogens, and five samples (21.7%) were positive for *Clostridium perfringens* enterotoxin. One sample (which was also positive for *C. perfringens* enterotoxin) was positive for yeast overgrowth with *S. cerevisiae*.

Discussion

At North Manchester General Hospital from March 2000 to April 2001 22.7% of cases of AAD were positive for CDT. This figure is similar to the findings of other workers,[2,16] but means that 75% of AAD is of unknown aetiology. However, during this study, a possible aetiological agent was found in 71% of cases, reducing undiagnosed cases by 48%.

Candida-associated diarrhoea is more common than *C. difficile*-associated diarrhoea in this study; an unexpected finding that lends weight to the argument that Candida-associated diarrhoea is a true clinical syndrome. However, it has treatment implications, as antifungal agents are required.

These results emphasise the importance of looking for candidal overgrowth in cases of AAD. The technique employed is sufficiently simple to be used on a daily basis in the enteric laboratory, and its introduction as a routine investigation when testing AAD samples should be considered.

The number of samples positive for *Candida spp.*

overgrowth in the present study is higher than that reported in comparable studies,[11,15] but this discrepancy may be due to the fewer numbers tested in the earlier studies. Furthermore, Danna *et al*.[11] excluded CDT-positive samples from their study. Results from North Manchester show that seven samples (11%) were positive for both CDT and *Candida* spp. overgrowth. These samples would not have been investigated for *Candida* spp. overgrowth by the criteria used by Danna *et al*., reducing the number of positive samples found in the earlier study.

Previous studies involved patients over the age of 50.[10,11] The mean age of patients in these studies were 70 and 74 years old, respectively. This compares well with the mean age (71) in the present study. Microscopy examination of AAD samples showed a reduction in normal faecal flora. This finding was not unexpected given the nature of antibiotic treatment received by this group and has been noted by other workers.[10,11]

Statistical analysis of the microscopy counts show there is good agreement between Gram film and quantitative culture for *Candida* spp. The Gram film proved to be a useful, rapid screening technique for AAD specimens, providing a rapid provisional diagnostic service. Cases could then be confirmed by quantitative culture.

Mycelial elements were seen in many of the Gram films from *Candida* spp.-positive samples. This supports the findings of other workers;[12] however, it cannot be assumed that mycelial elements are linked to diarrhoea.

When samples were of a more solid consistency, serial dilutions were used to quantify *Candida* spp. Formed stool samples did not reveal counts $>10^5$ cfu/g. The 2-µL loop technique compared well with serial dilution, and some samples that were loose or fluid in consistency had both quantification techniques applied. The 2-µL loop technique has the advantage of being less demanding technically and as sensitive as serial dilution; however, serial dilution provides an exact count.

Two *Candida* spp. (*C. albicans* and *C. glabrata*) were isolated in relatively equal numbers. Typing results showed that the strains were not related epidemiologically, suggesting that *Candida* spp. overgrowth is an endogenous infection and poses no infection control risks. It is the *Candida* sp. present in the normal faecal flora that proliferates and overgrows after antibiotic therapy, causing diarrhoea.

One sample was positive for overgrowth with *S. cerevisiae*. Alternative treatment regimes involving the use of *S. cerevisiae* to recolonise the intestine[17] may offer an explanation for this; however, no mention of such biotherapy could be found in the clinical information gathered on this patient.

Clostridium difficile

Clostridium difficile cytotoxin B was detected in 34.9% of AAD samples, which was a higher rate than reported normally (approximately 22%) at North Manchester General Hospital. This may be because the criteria used for testing samples were adhered to more rigidly in this study. Generally, samples requesting CDT testing are screened by the control of infection staff. Results of this study emphasise the importance of adherence to testing guidelines.

Clostridium perfringens

Samples positive for *C. perfringens* enterotoxin accounted for the lowest number of positives (9.5%). Prevalence of *C. perfringens* diarrhoea (non-food related) seems to vary considerably from study to study. Incidence rates of 5% to 25% have been reported.[5,18] The rate found in the present study falls between these figures. It has been suggested that differences in *C. perfringens*-positive rates may be due to the population tested.[18]

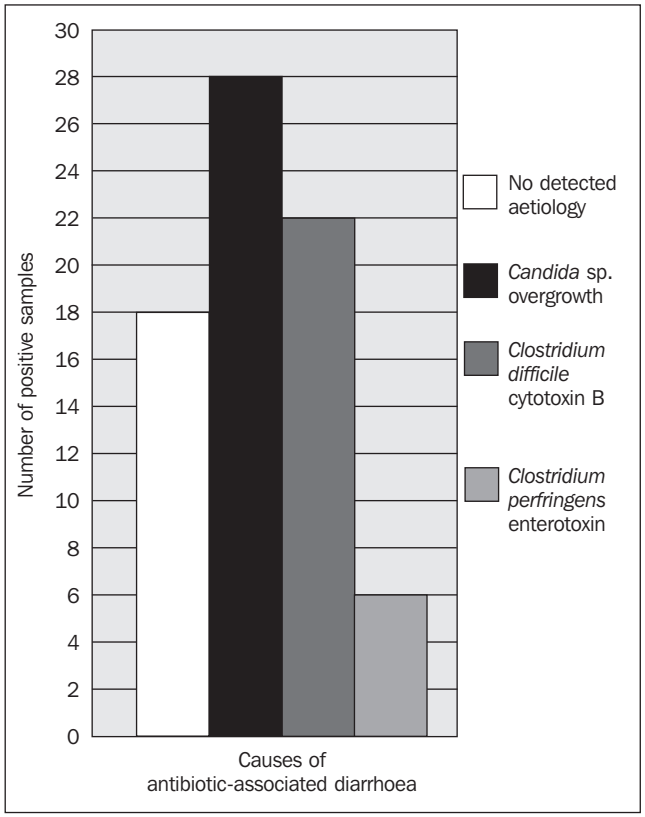

Fig. 1. Aetiology of antibiotic-associated diarrhoea.

Negative AAD samples

Some samples (29%) proved to be negative for all three investigated aetiologies. It was interesting to note that two out of the 18 negative samples showed a heavy growth of *Pseudomonas* sp. and no normal faecal flora. This may have been an overgrowth and as such a cause of diarrhoea. It has been stated that clinically mild cases of AAD may be due to non-infectious causes such as disturbance in carbohydrate metabolism[19] or an allergic reaction to antibiotics. Two out of the 18 samples grew no bacterial flora, and this elimination of the normal flora is enough to cause diarrhoea.[20]

Control group 1

The average age (42 years) of those in control group 1 was much lower than the average age of the AAD patient group. It is well documented that age is a significant risk factor in the development of AAD.[9,11,21] All samples from this group were negative for all investigations and yeast counts were all below 10^5 cfu/g. This supports the theory that although *Candida* spp. are present in the normal faecal flora they are not present at levels seen in patients with AAD.

Control group 2

The average age of those in control group 2 was much closer

to that of the AAD patient group. Only one sample showed *Candida* sp. overgrowth. However, although these people had diarrhoea, they did not show the same level of *Candida* spp. overgrowth or number of CDT-positive samples. These findings suggest that both Candida-associated diarrhoea and *Clostridium difficile*-associated diarrhoea occur in hospital in-patients. This lends weight to the argument that it is the hospital environment (i.e., administration of numerous broad-spectrum antibiotics) that promotes a different cause of diarrhoea.

In conclusion, *Candida* spp. overgrowth and its association with AAD is largely overlooked and undiagnosed. This study shows that at North Manchester General Hospital candidal overgrowth may be responsible for more cases of AAD than is *Clostridium difficile*.

Gram staining and quantitative culture of faecal specimens is a rapid, inexpensive and technically undemanding diagnostic procedure. Samples received for CDT testing should also be considered for quantitative *Candida* spp. culture. If both tests are negative and diarrhoea persists then other infectious agents (e.g., *Clostridium perfringens*) should be investigated.

The vast majority of patients with AAD studied here were on multiple 'high risk' antibiotic regimes. Medical staff should consider whether or not such broad-spectrum treatment is necessary before it is given and alternative therapies should always be considered. Intelligent prescribing combined with basic hygiene, infection control procedures and education can further reduce the number of patients suffering from this debilitating, sometimes fatal hospital-acquired disorder.

Finally, work involving controlled trials of nystatin therapy should be considered, as other workers have reported successful use of antifungal treatment.[10,11]

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