Isolation of *Clostridium difficile* from human jejunum: identification of a reservoir for disease?

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SUMMARY The possibility that the small intestine may represent a reservoir for *Clostridium difficile* was studied, using segments of human jejunum collected at necropsy. Our results (three of 100 specimens positive for *C difficile* culture) support the hypothesis that *C difficile* can be found in human jejunum and that it adheres to the normal mucosa as a resident bacterium. These findings suggest that gastrointestinal disease caused by *C difficile* has an endogenous origin.

The possibility that the small intestine might represent a reservoir for disease caused by *Clostridium difficile* was suggested by Taylor *et al*, when they isolated *C difficile* from a jejunal aspirate of a patient with chronic colitis.¹ This hypothesis was confirmed by our experience with a case of pseudomembranous enteritis with spared colon, in which we isolated *C difficile* from the patient's ileum obtained at necropsy.²

To elucidate these findings we carried out a study to verify the rate of isolation of *C difficile* from human small intestine using segments of jejunum that had been obtained at necropsy.

Material and methods

COLLECTION OF SPECIMENS

Over six months one hundred segments of proximal jejunum were collected within 48 hours from 100 patients who had died. The specimens were about 10 cm long and macroscopically free from lesions. Each segment was placed in a sterile Petri dish and immediately sent to the bacteriology laboratory.

The subjects had died from different diseases, none of them had had diarrhoea or other gastrointestinal symptoms in life. The mean age was 70 years (range 52-86); 90% of the patients had received treatment with antibiotics—that is, β -lactam antibiotics alone, or in conjunction with aminoglycosides.

PROCESSING OF THE SPECIMENS

To remove the bacteria that were not firmly attached to mucosa each segment was carefully washed with 10 cc of a sterile saline solution using a vortex mixer for 10 minutes.³ This procedure was repeated three

times for each sample, changing the container and the washing liquid each time. After this the segment was stretched and the mucosa removed with a sterile lancet; the material obtained was used to inoculate a cycloserine-cefoxitin-fructose selective agar (CCFA) plate.⁴ The plates were screened for colonies characteristic of *C difficile*; all the cultures were incubated for at least five days before being discarded.

The three washings from each segment were centrifuged for 10 minutes at 5000 rpm and the sediments were used to inoculate a CCFA plate.

Results

Within 48 hours the cultures from the mucosa were positive for *C difficile* in three cases. Prolonged incubation of the other samples did not yield any additional positive results. None of the centrifuged washings yielded *C difficile*. The ages of the culture positive patients were 63, 60, and 74 years; all of them had received treatment with antibiotics.

Discussion

Although the aetiological role of *C difficile* in pseudomembranous colitis is now widely accepted, there is still some controversy about the transmission of the disease.

The low percentage of isolation from faeces of healthy adults,⁵ the description of small outbreaks,⁶⁻⁸ and the isolation of *C difficile* from animal reservoirs⁹ favour an exogenous origin of the disease rather than an antibiotic induced overgrowth of undetectable amounts of *C difficile* that may be already present in the normal intestinal flora.

The isolation of *C difficile* from a jejunal aspirate of a patient with chronic diarrhoea by Taylor *et al* and

the case report of *C difficile* pseudomembranous enteritis (with large intestinal sparing) that we observed,² made us consider that the jejunum could be a reservoir for disease.

Our results support the hypothesis that *C difficile* can be found in the jejunum and in subjects without gastrointestinal change; furthermore, the absence of *C difficile* from washings of anatomical segments indicates that *C difficile*, when present, adheres to the normal mucosa, which facilitates its integration into the resident flora. These findings suggest an endogenous origin for *C difficile* mediated gastrointestinal disease.

Interestingly, the rate of isolation (3%) found in our work was comparable with that of the isolation of *C difficile* from the faeces of healthy adults; this raises the possibility that carriers may be colonised throughout the whole of the intestinal tract. To confirm this hypothesis additional studies are required that will evaluate the colonisation of different parts of the intestine by *C difficile* in the same subject.

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