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Treatment of Relapsing *Clostridium difficile* Diarrhoea by Administration of a Non-Toxigenic Strain

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Two patients with relapsing *Clostridium difficile* diarrhoea following metronidazole and vancomycin therapy were colonised with a non-toxigenic avirulent *Clostridium difficile* strain given orally in three doses. Both patients appeared to respond without side-effects. Oral bacteriotherapy with a defined non-toxigenic strain of *Clostridium difficile* would appear to represent an acceptable, alternative and novel way to treat hospitalised patients who relapse with *Clostridium difficile* diarrhoea after specific antibiotic therapy.

Relapsing diarrhoea due to toxin-producing *Clostridium difficile* following specific therapy with vancomycin was first described by Bartlett et al. and Fekety et al. five years ago (1, 2), but the problem, which affects up to 20% of patients, remains unsolved. The reinfection that occurs may be from an exogenous source or represent recurrence from an endogenous source. Wilson et al. (3) have shown that caecitis can be prevented in antibiotic treated hamsters by giving them normal caecal flora by a combination of the oral and rectal routes, and Bowden et al. (4) and Schwan et al. (5) have successfully used faecal enemas to treat humans. There is obviously some concern with giving patients a complex, mixed, undefined flora which will contain a number of potential pathogens. Borriello and Barclay (6) have shown that hamsters can be protected against developing *Clostridium difficile* diarrhoea by prior colonisation with non-toxigenic *Clostridium difficile*. On the basis of their study, we investigated the possible therapeutic effect of one of these well-defined avirulent strains of *Clostridium difficile*, which fails to produce either cytotoxin (toxin B) or enterotoxin (toxin A) in two patients who each relapsed twice.

The first patient (patient A), aged 88 years, had received cotrimoxazole and cephalexin for a catheter-associated urinary tract infection prior to developing *Clostridium difficile* diarrhoea. A course of oral metronidazole (400 mg three times daily) was given successfully. However, the patient relapsed within two days and was then treated with oral vancomycin (125 mg four times daily). Two weeks following vancomycin, diarrhoea recurred with *Clostridium difficile* and its cytotoxin present in the stool, so that the patient was again treated with vancomycin. Although diarrhoea ceased with the second course of vancomycin, *Clostridium difficile* persisted in her stool for at least three days following therapy. Due to the patient's history of relapse there was concern that relapse might re-occur, and treatment was therefore commenced with a non-toxigenic strain of *Clostridium difficile* at a time when toxigenic strains of *Clostridium difficile* were still being excreted. The second patient (patient B), aged 76 years, had received ampicillin and cephalexin for a urinary tract infection prior to developing *Clostridium difficile* diarrhoea. A course of metronidazole (400 mg four times daily) was given but diarrhoea recurred after eight days when oral vancomycin (125 mg four times dally) was given. Twenty days later the patient relapsed with further diarrhoea due to *Clostridium difficile*. Another course of oral vancomycin (125 mg four times daily)

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resulted in cessation of diarrhoea, although *Clostridium difficile* persisted in her stool. She was then given an oral dose of the non-toxigenic *Clostridium difficile* strain on three successive days.

Stool samples from both patients were cultured for salmonella, shigella and campylobacter by standard laboratory techniques, as well as for *Clostridium difficile* using a selective agar (7). Three isolates of *Clostridium difficile* from the highest dilution were sub-cultured and tested for production of cytotoxin (toxin B) in vitro. In addition, a ten-fold dilution of the faecal specimen in Brain-Heart Infusion (BHI) broth (Difco, UK) incorporating cycloserine and cefoxitin, was incubated anaerobically at 37 °C for three days and tested for cytotoxin to detect the presence of smaller numbers of toxigenic strains. *Clostridium difficile* cytotoxin was assayed in VERO cell cultures using a cover-slip technique (8). Cytotoxic effects were confirmed to be due to *Clostridium difficile* toxins by neutralization with the cross-reacting *Clostridium sordellii* antitoxin.

A non-toxigenic strain of *Clostridium difficile* (M-1) which had previously been shown to protect hamsters (6) was used for bacteriotherapy. It was cultured in BHI for 24 h at 37 °C under anaerobic conditions. One ml was suspended in approximately 50 ml of milk to yield approximately 10^7 CFU of *Clostridium difficile*/ml. This was given to each patient as a single oral dose on three successive days. Both patients were given an H_2 antagonist (ranitidine 150 mg twice daily) one day prior to and during bacteriotherapy.

Non-toxigenic *Clostridium difficile* persisted in the stool of patient A for up to 14 days after the oral dose was given. Toxigenic *Clostridium difficile* and its cytotoxin had disappeared from the stool specimen collected immediately prior to bacteriotherapy and were not detected in her stool thereafter. She has not relapsed for the last four months but has had problems with constipation. Results of stool cultures and toxin assays from patient B are given in Table 1. During the first three days following the start of bacteriotherapy toxigenic *Clostridium difficile* and its cytotoxin were still present in the stool but after six days the cytotoxin was absent and the non-toxigenic strain had established itself to levels of 10^7 CFU/g of stool. Seventeen days after the commencement of bacteriotherapy she had a two day episode of mild self-limiting diarrhoea, when toxigenic *Clostridium difficile* and cytotoxin were again present. This was in marked contrast to previous relapses when diarrhoea continued unabated. Both strains were present between 17 and 28 days but then disappeared together from the stool between 28 and 45 days later – a more accurate determination could not be achieved due to the patient's constipation. The patient died one month later due to an unrelated cause but had experienced no further diarrhoea.

Table 1: Presence of *Clostridium difficile* and toxin B in patient B after an oral dose of a non-toxigenic strain.

Days after oral dose	No. of organisms (log_{10})/g stool		Faecal toxin B
	Toxigenic	Non-toxigenic	
1	7.5	negative	positive
2	7.0	negative	positive
3	5.9	negative	positive
6	7.4	7.1	negative
9	5.0	negative	negative
10	5.8	negative	negative
17[a]	3.2	3.5	positive
24	negative	3.8	negative
28	2.3	2.6	negative
45	negative	negative	negative

[a] Two-day episode of diarrhoea.

We have previously considered bacteriotherapy as an alternative way of treating *Clostridium difficile* infection (6, 9). We have now found that a non-toxigenic avirulent strain of *Clostridium difficile* can be administered safely by the oral route and that it will colonise the gastrointestinal tract for up to 28 days post-therapy. Although it was given to the two patients after diarrhoea had ceased, they did not relapse as occurred previously following specific antibiotic therapy.

The second patient had toxigenic *Clostridium difficile* present when the non-toxigenic strain was given and carriage ceased in competition with it. While it is not certain in either patient that the bacteriotherapy actually prevented relapse, we suggest it contributed to the satisfactory outcome.

Although crude preparations of faeces containing a complex mixed undefined flora have been used with some success (4, 5) in a bacteriotherapeutic approach in the treatment of gastrointestinal disease due to *Clostridium difficile*, there are associated aesthetic problems and dangers of infecting the patient with other pathogens. Our findings indicate that the use of avirulent non-toxigenic strains of *Clostridium difficile* may be a much more acceptable alternative. There is now a need for prospective studies with this type of therapy in the management of relapsing *Clostridium difficile* diarrhoea in hospital, especially in those patients who are particularly susceptible such as the elderly and the immunosuppressed. Specific antibiotic therapy needs to be compared with bacteriotherapy, both alone and in combination. If bacteriotherapy can be shown to prevent relapse in a larger study, then this would indicate that trials should be considered for it as first-line treatment for *Clostridium difficile* diarrhoea in hospital.

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Multiply Resistant *Streptococcus mitis* Isolated from Conjunctival Exudate of Newborns

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The isolate had group C streptococcal antigen, was beta-haemolytic on horse but alpha-haemolytic on sheep blood agar plates and might therefore be misidentified as beta-haemolytic group C streptococci.

In addition to beta-haemolytic streptococci of groups A, B and D, other beta-haemolytic streptococci and members of the viridans group are frequently isolated from human infections. Most of the isolates are universally sensitive to various antimicrobial agents including penicillins, cephalosporins and erythromycin. However, streptococci resistant to one or more antimicrobial agents commonly used are occasionally found (1–3). We report on multiply resistant streptococci isolated from conjunctival exudate from seven newborns (age 2–7 days) in three different obstetric wards in one hospital.

Samples transported in Stuart tubes (Transpocult, Orion Diagnostica, Finland) were cultured onto 5 % horse blood, chocolate and Gonococcus Selective (Gibco Ltd., Scotland) agar plates. The plates were incubated at 35° in an atmosphere of 5 % CO_2 in air for 24–48 h. In addition to the streptococci, *Staphylococcus epidermidis* and/or *Staphylococcus aureus* but no gonococci grew in the conjunctival samples.

The isolated strain of *streptococcus* sp. had the following characteristics. Small colonies were surrounded by large beta-haemolytic zones on horse blood agar routinely used in the laboratory. The haemolysis was the same regardless of whether the plates were grown in air, 5 % CO_2 or anaerobically. However, when tested in a reference laboratory on sheep blood agar plates the isolate was found to be alpha-haemolytic. The strain was bile-aesculin negative, bacitracin and optochin resistant and was positive only for group C streptococcal antigen both by Streptex (Wellcome Diagnostics, UK) and Streptococcal Grouping Kit (Oxoid Ltd., UK). Tested on API 20 Strep (Api System, France) the streptococci were positive for alkaline phosphatase and leucine arylamidase but negative for beta-glucuronidase and Voges-Proskauer (VP) reaction. They produced acid only from lactose. The biochemical profile obtained by the API 20 Strep was 0060400, which identified the isolate as *Streptococcus mitis* provided it was alpha-haemolytic not beta-haemolytic. Its biochemical characteristics are given in Table 1.

When tested by the disc diffusion method (Rosco Neo-Sensitabs, Rosco Diagnostica, Denmark) on Mueller-Hinton II (BBL Microbiology System, USA) agar enriched with 1 % haemoglobin (Oxoid Ltd., UK) and 1 % Iso Vitalex (BBL Microbiology System, USA) added, the isolates were resistant or had reduced sensitivity to a number of commonly used antimicrobial agents (Table 2), but sensitive to cefotaxime, clindamycin, vancomycin and rifampicin. The same pattern was observed when minimal inhibitory concentrations

***Streptococcus mitis* resistant to most of the commonly used antimicrobial agents but sensitive to cefotaxime, clindamycin, vancomycin and rifampicin was isolated from conjunctival exudate of newborns.**

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