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Antimicrobial susceptibilities of *Clostridium difficile*

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SUMMARY The antimicrobial susceptibilities of 78 strains of *Clostridium difficile* isolated from patients with and without gastrointestinal symptoms were determined and compared. Strains from patients with symptoms were more likely to show resistance to antibiotics. The antimicrobial susceptibilities of toxigenic and non-toxigenic strains were found to be similar.

In the last two years advances have been made in understanding the aetiology of pseudomembranous colitis (PMC). Larson and Price[1] and Rifkin *et al.*[2] demonstrated a toxin in the faeces of patients with PMC that was shown to be neutralised by *Clostridium sordellii* antitoxin and produced by *Clostridium difficile*.[3] More evidence has accumulated[4 5] that suggests that *Cl. difficile* is associated with antibiotic-associated PMC. This condition has been described in patients who have received treatment with an antibiotic from most of the major groups of antibiotics except vancomycin and metronidazole. The presence of the toxin of *Cl. difficile* in faeces has also been associated with diarrhoea related to antibiotic therapy, but without the pathological features of PMC. It is thought that the antibiotic therapy produces changes in the faecal flora and allows *Cl. difficile* to proliferate and produce pathological amounts of toxin. It is therefore of interest to establish the antibiotic sensitivity patterns of *Cl. difficile* isolated from the faeces of normal individuals.

All strains of *Cl. difficile* isolated from patients with PMC have been found to be highly sensitive to vancomycin,[6 7] and vancomycin has been used with success in the treatment of PMC,[8 9] though relapse has been observed after cessation of therapy.[10] Published reports[6 7] on the antibiotic susceptibility of *Cl. difficile* have given data on only a few strains, and these have been collected mainly from proven cases of PMC. This report gives data on a total of 78 strains, including those isolated from patients without gastrointestinal symptoms. A comparison has been made of the sensitivity patterns of both toxigenic and non-toxigenic strains, and the susceptibilities of strains isolated from asymptomatic carriers have been compared with those from patients with gastrointestinal symptoms.

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Material and methods

SOURCE OF STRAINS

Seventy-two strains of *Cl. difficile* were isolated from the faeces of patients with PMC or antibiotic-associated diarrhoea and from faeces of patients without gastrointestinal symptoms (Table 1). Not all patients with symptoms were subjected to sigmoidoscopy so we have regarded patients with PMC or antibiotic-associated diarrhoea as a single group. In addition, strains 17249, 7988, 32288, 13231, 25917, and 21153 were supplied by Dr DW Burdon, of Birmingham; all six were toxigenic strains isolated from adults with PMC. The organisms were identified on the grounds of colonial and cellular morphology, biochemical reactions, and gas-liquid chromatography.[11]

Table 1 *Source of strains of* Cl. difficile

Patient category	*Toxigenic*	*Non-toxigenic*
Symptomless babies 0-2 years	29	31
Symptomless adults	0	1
Symptomatic adults, Manchester	10	1
Symptomatic adults, Birmingham	6	0

TOXIGENICITY TESTING

Cultures of *Cl. difficile* were grown in cooked meat medium for two days at 37°C, and culture supernatants were tested for cytopathogenicity to MRC 5 cells and neutralisation by *Cl. sordellii* antitoxin.[1] During the course of toxigenicity testing it was found that some strains failed to show a cytopathic effect. In order to confirm the absence of toxigenicity, one such strain was subjected to further testing. The

organism was grown in a medium containing 2% Proteose Peptone No. 3 (Difco) and 1% glucose, and duplicate cultures were incubated at 37°C and 42°C for three days before being tested for cytopathogenicity in tissue culture, as described above, with negative results. The same strain was then cultured for two days in Brain-Heart Infusion broth (Oxoid) at 37°C, and 0·5 ml of the culture supernatant was injected subcutaneously into a guinea-pig, as described by Hall and O'Toole.[12] The guinea-pig failed to show any signs of toxaemia. This strain and eight other 'non-toxigenic' strains were grown in 2% Proteose Peptone No. 3 (Difco) containing 0·5% glucose and 1 μg/ml clindamycin for three days and retested for toxin production in tissue culture. The presence of this concentration of clindamycin did not stimulate these strains to produce toxin. Subsequently, strains that failed to show a cytopathic effect were regarded as non-toxigenic.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS (MIC)

All strains were tested against the following antibiotics by the agar dilution method: ampicillin, cefoxitin, cephalexin, clindamycin, metronidazole, erythromycin, sulphadiazine, tetracycline, and vancomycin. The antibiotics were incorporated in doubling dilutions in 20 ml DST agar plates containing 10% lysed horse blood, giving final concentrations ranging from 0·125 to 1024 μg/ml. Suspensions of *Cl. difficile* from overnight cultures on blood agar were made in nutrient broth and applied to the agar surface using a multipoint inoculator. The inoculum was of the order of 10^4 colony-forming units. All plates were incubated anaerobically for 48 hours at 37°C using the Gas Pak system (Becton Dickinson Ltd).

DISC SENSITIVITY TESTS

Disc sensitivity tests were performed on Oxoid DST agar containing 10% lysed horse blood incubated at 37°C under anaerobic conditions. Results were recorded after 18-24 hours' incubation. The following antibiotic discs were used: tetracycline (10 μg), erythromycin (5 μg), clindamycin (2 μg), ampicillin (10 μg), cephradine (30 μg), cotrimoxazole (25 μg), chloramphenicol (30 μg), vancomycin (5 μg), metronidazole (5 μg), fucidin (10 μg), colistin (10 μg), gentamicin (10 μg), novobiocin (5 μg), spectinomycin (25 μg), trimethoprim (1 μg), and nalidixic acid (30 μg).

Results

MINIMUM INHIBITORY CONCENTRATIONS

The results of MIC determinations are given in

Table 2 *Minimum inhibitory concentrations for various antibiotics (78 strains)*

*Group**	*Antibiotic*	*No. of strains with MIC (μg/ml) of:*												
		≤0·125	*0·25*	*0·5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*	*128*	*256*	*≥1024*
I	Cefoxitin	—	—	—	—	—	—	—	—	—	1	28	—	—
II		—	—	—	—	—	—	—	—	1	22	9	—	—
III		—	—	—	—	—	—	—	—	—	3	12	2	—
I	Cephalexin	—	—	—	—	—	—	—	—	—	1	15	13	—
II		—	—	—	—	—	—	—	—	—	13	15	4	—
III		—	—	—	—	—	—	—	—	—	1	4	12	—
I	Vancomycin	—	—	—	10	13	6	—	—	—	—	—	—	—
II		—	—	—	26	5	1	—	—	—	—	—	—	—
III		—	—	—	2	10	5	—	—	—	—	—	—	—
I	Erythromycin	—	—	22	7	—	—	—	—	—	—	—	—	—
II		—	1	28	3	—	—	—	—	—	—	—	—	—
III		—	3	8	—	—	1	1	—	1	—	—	—	3
I	Ampicillin	—	—	—	—	12	17	—	—	—	—	—	—	—
II		—	—	—	—	7	25	—	—	—	—	—	—	—
III		—	—	—	2	1	12	2	—	—	—	—	—	—
I	Clindamycin	—	—	—	1	5	22	1	—	—	—	—	—	—
II		—	—	—	—	3	26	3	—	—	—	—	—	—
III		—	—	—	—	4	8	—	—	—	—	—	—	5
I	Tetracycline	2	23	3	1	—	—	—	—	—	—	—	—	—
II		1	24	3	3	—	1	—	—	—	—	—	—	—
III		3	5	3	—	—	—	—	—	—	5	1	—	—
I	Metronidazole	—	—	11	11	—	7	—	—	—	—	—	—	—
II		—	—	15	9	1	7	—	—	—	—	—	—	—
III		1	—	6	8	1	1	—	—	—	—	—	—	—
I	Sulphadiazine	—	—	—	—	—	1	12	8	8	—	—	—	—
II		—	—	—	—	—	3	19	8	2	—	—	—	—
III		—	—	—	—	2	—	4	8	2	—	—	—	1

*Group I 29 toxigenic strains of *Cl. difficile* from babies aged 0-2 years.
II 32 non-toxigenic strains of *Cl. difficile* (31 from babies aged 0-2 years and 1 from a symptomless adult).
III 17 strains of *Cl. difficile* from adults with gastrointestinal symptoms (including 1 non-toxigenic strain).

Table 3 *Antibiotic susceptibilities of strains from patients with gastrointestinal symptoms*

Patient No.	*Previous antibiotic treatment*	*Sensitivity pattern*
M1*	None	Resistant to erythromycin, clindamycin†
M2	Cotrimoxazole	Resistant to tetracycline, cotrimoxazole
M3	Ampicillin, flucloxacillin	Typical‡
M4	Ampicillin	Typical
M5	Clindamycin	Resistant to tetracycline, erythromycin, clindamycin
M6	Gentamicin, flucloxacillin	Typical
M7	Cotrimoxazole, flucloxacillin, gentamicin, carbenicillin	Typical
M8	Cephalexin	Typical
M9	Cephradine, gentamicin, ampicillin	Typical
M10	Ampicillin, metronidazole, cotrimoxazole	Resistant to tetracycline, erythromycin
M11	Clindamycin	Resistant to tetracycline, erythromycin, clindamycin
B1	Not known	Resistant to erythromycin, clindamycin
B2	Not known	Typical
B3	Not known	Resistant to tetracycline
B4	Not known	Resistant to tetracycline, erythromycin, clindamycin
B5	Not known	Typical
B6	Not known	Typical

*M = Manchester; B= Birmingham.
†This strain, isolated from a patient with diarrhoea, failed to produce detectable amounts of toxin.
‡Typical = resistant to cephalexin, cefoxitin, gentamicin; moderately resistant to clindamycin; and sensitive to tetracycline, erythromycin, cotrimoxazole.

Table 2. The MIC results were in accord with the disc sensitivity results except for cephalexin, cefoxitin, and ampicillin. All strains were resistant (MIC 32-256 μg/ml) to both cephalexin and cefoxitin, and the MIC of ampicillin varied between 1 and 8 μg/ml. All strains were sensitive to metronidazole (MIC 0·125-4 μg/ml) and vancomycin (MIC 1-4 μg/ml). The susceptibilities of the strains isolated from patients with symptoms were compared to previous antibiotic therapy (Table 3).

DISC SENSITIVITY TESTS

Disc sensitivity tests correlated well with the MIC results for the antibiotics given in Table 2. In addition, all strains gave zones greater than 20 mm with fucidin, and no zones were developed against colistin, gentamicin, novobiocin, spectinomycin, trimethoprim, or nalidixic acid. Two strains showed smaller than average zones with chloramphenicol; both were from cases of PMC and both were from Birmingham.

Discussion

It is not clear from published work whether the strains associated with gastrointestinal symptoms differ in their antibiotic susceptibilities from those of strains isolated from symptomless excreters. All the strains of *Cl. difficile* that we examined were resistant to cephalexin and cefoxitin. Resistance to clindamycin, tetracycline, erythromycin, or cotrimoxazole was observed but was restricted to strains isolated from patients with gastrointestinal symptoms. It may be that the use of any of these agents could be associated with the proliferation of *Cl. difficile* in the gut. There were no common combinations of antibiotic resistance among the strains isolated from patients with symptoms although, as shown in Table 3, many were resistant to an antibiotic previously taken by the patient. For the majority of strains, the MIC of clindamycin was 1-8 μg/ml, but those strains that showed no zones on disc testing were much more resistant (MIC > 1024 μg/ml), and all of these came from patients with gastrointestinal symptoms. All the strains of *Cl. difficile* were uniformly sensitive to vancomycin, confirming the conclusions of Burdon *et al.*[7] that this drug may be expected to produce good results for the treatment of PMC. There was no correlation between the production of toxin and resistance to antibiotics since the patterns for toxigenic and non-toxigenic strains from symptomless excreters were generally similar. Indeed, five asymptomatic patients yielded both toxigenic and non-toxigenic strains, and when the susceptibilities of these pairs of strains were compared there were no significant differences.

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