

## In vitro susceptibility of rabbit strains of *Clostridium spiroforme* to antimicrobial agents

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### ABSTRACT

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Using an agar dilution method we measured the minimum inhibitory concentration (MIC) of 12 antimicrobial agents against 11 strains of iota-toxigenic strains of *Clostridium spiroforme*. Each strain was isolated from a separate outbreak of toxic diarrhoea of rabbits. Vancomycin and bacitracin, both agents used to treat intestinal clostridiosis of humans and other animals, had a relatively high MIC (8 µg/ml or more). Metronidazole was uniformly active against *C. spiroforme*. With MIC of 8 µg/ml or more, both lincomycin (11 strains) and erythromycin (9 strains) were relatively inactive against *C. spiroforme*; conversely, penicillin G was active (MIC for 8 strains was 0.5 µg/ml or less). Exposure to any one of these drugs has been implicated as a predisposing factor for *C. spiroforme* mediated diarrhoea of rabbits. The greatest variation in MIC was seen for erythromycin (8-fold), penicillin G (8-fold) and tetracycline (16-fold).

### INTRODUCTION

*Clostridium spiroforme* is a toxin producing bacterium causing diarrhoea of rabbits. Its iota-like toxin is neutralized by antiserum raised against the iota toxin of *Clostridium perfringens* Type E (Carman and Borriello, 1982a,b; Borriello and Carman, 1983). It has also been found in the faeces of other lagomorphs (Carman and Evans, 1984) and human (Babudieri et al., 1986). The bacterium causes an infectious enterotoxaemia characterized by diarrhoea and death (Carman and Borriello, 1984). Iota-like toxin is always present in caecal supernatant fluid. The predisposing factors for *C. spiroforme* mediated diarrhoea are weaning, exposure to antimicrobial agents and "stresses" such as lactation, old age and dietary change (Carman and Evans, 1984).

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We have examined 11 strains of *C. spiroforme*, each from a different outbreak of diarrhoea, for their susceptibility to antimicrobial agents. Some of these antibiotics are used to treat clostridial infections of the gastrointestinal tracts of humans and other animals; others were selected because of their earlier promise as medium supplements for the selection of this species (unpublished results). Occasionally apparently healthy rabbits have been shown to be carrying *C. spiroforme* shown to be toxigenic in vitro (Borriello and Carman, 1983). This represents either the early stages of infection (Carman and Borriello, 1988) or the asymptomatic carriage of the bacterium. A good selective medium would help resolve this issue, possibly identifying animals, refractory to the toxin, which represent a reservoir for infecting susceptible individuals. We therefore tested *C. spiroforme* for antimicrobial susceptibility with the eventual aim of developing a selective medium. The list of drugs tested also includes some agents that cause the antibiotic-associated form of the disease (Carman and Evans, 1984) by breaking down the colonization resistance of the rabbit commensal gut flora.

#### MATERIALS AND METHODS

*Bacterial strains.* Eleven strains of *C. spiroforme* were used in this study (Table 1). They were isolated from unrelated outbreaks of diarrhoea amongst farm and laboratory rabbits (*Oryctolagus cuniculus*; 10 strains) and a captive eastern cottontail (*Sylvilagus floridanus*; 1 strain).

TABLE 1  
Sources of *Clostridium spiroforme*

Strain	Origin	Reference
NCTC 11493	Laboratory rabbit: London, UK	1,2,3
A 7	Farm rabbit: Normandy, France	1,2,3
A 8		
B 61		
C 12		
D 11		
D 13		
D 14		
CDE 1	Laboratory rabbit: UK	3,4
RPO 980	Laboratory rabbit: Dr. Richard Orcutt, Charles River, Inc., Wilmington, DE, USA	3,5
B 61	Captive Eastern Cottontail: Dr. Richard Evans, Treetops Wildlife Refuge, Brighton, IL, USA	
References:	1: Carman and Borriello (1982a) 2: Carman and Borriello (1982b) 3: Borriello and Carman (1983) 4: Baskerville, Wood and Seamer (1980) 5: Orcutt, Foster and Jonas (1978)	

*Antimicrobial agents.* The agents tested were: bacitracin, carbenicillin, chloramphenicol, erythromycin, kanamycin, lincomycin, metronidazole, nalidixic acid, penicillin G, rifampicin, tetracycline and vancomycin. Details of the sources of each are given in the acknowledgements.

*Procedures.* The methods employed were those described by Zabransky et al. (1985), with only minor changes. Briefly, the bacteria were grown in thioglycollate broth with added haemin (5 µg/ml) and menadione (0.1 µg/ml) for 8 h anaerobically at 37°C. Two-fold dilutions of each test compound were prepared freshly in Wilkins-Chalgren agar (Wilkins and Chalgren, 1976). The inocula were adjusted to a turbidity equal to that of a 0.5 MacFarland standard (Zabransky et al., 1985) and applied to the surface of the agar plates using a Steer's replicator. Once inoculated, the plates were incubated in an anaerobic glove box (80% nitrogen, 10% hydrogen and 10% carbon dioxide) at 37°C for 48 h. The minimum inhibitory concentration (MIC) was the

TABLE 2

Minimal inhibitory concentrations of antimicrobial agents for strains of *Clostridium spiroforme*

Strain	Minimum inhibitory concentration <sup>1</sup>											
	Bac	Carb	CM	Eryt	KN	LN	MZ	NAT	PenG	RIF	Tet	VN
<i>Clostridium spiroforme</i>												
A7	>8	4	4	>8	8	>8	0.125	>8	0.25	>8	>8	8
A8	>8	4	4	>8	>8	>8	0.125	>8	0.25	>8	>8	8
BA1	>8	8	2	1	>8	>8	0.25	>8	0.5	>8	>8	8
B61	>8	4	4	>8	8	>8	0.125	>8	0.25	>8	>8	8
C12	>8	2	4	>8	8	>8	0.125	8	0.125	>8	>8	8
D11	>8	8	4	>8	8	>8	0.125	>8	1	>8	>8	8
D13	4	2	4	>8	8	>8	0.125	>8	0.25	>8	>8	8
D14	>8	8	4	>8	8	>8	0.063	>8	0.5	>8	0.5	>8
CDE 1	>8	4	2	>8	>8	8	0.063	>8	1	>8	1	4
RPO 980	8	>8	2	1	>8	8	0.125	>8	1	>8	0.5	4
NCTC 11493	8	4	4	>8	>8	>8	0.063	>8	0.5	>8	0.5	4
Control <sup>2</sup> organisms:												
ATCC 13124	0.25	0.125	2	2	>8	0.5	0.125	>8	0.063	0.016	0.125	1
ATCC 25285	>8	>8	4	>8	8	>8	0.25	2	>8	>8	0.25	8
ATCC 25922	>8	>8	4	>8	8	>8	>8	2	>8	>8	2	8
ATCC 25923	>8	1	8	0.5	4	0.25	>8	>8	0.032	0.016	0.125	4

<sup>1</sup>Expressed in µg/ml except for penicillin G which was in Unit/ml.

<sup>2</sup>ATCC 13124 = *Clostridium perfringens*

ATCC 25922 = *Escherichia coli*

ATCC 25285 = *Bacteroides fragilis*

ATCC 25923 = *Staphylococcus aureus*

Bac = bacitracin, Carb = carbenicillin, CM = chloramphenicol, Eryt = erythromycin, KN = kanamycin, LN = lincomycin, MZ = metronidazole, NAT = nalidixic acid, PenG = penicillin G, RIF = rifampicin, Tet = tetracycline, VN = vancomycin.

lowest concentration of antimicrobial agent permitting no macroscopic growth. Each assay was done three times.

## RESULTS

The 11 test strains were relatively resistant to nalidixic acid, lincomycin, kanamycin and rifampicin (MIC of 8  $\mu\text{g}/\text{ml}$  or greater) (Table 2). Bacitracin and vancomycin were only slightly less active (4 to  $> 8 \mu/\text{ml}$ ). All strains were sensitive to metronidazole (0.063 to 0.25  $\mu\text{g}/\text{ml}$ ) and penicillin G (0.125 to 1 U/ml). Generally the strains had an intermediate sensitivity to carbenicillin (2 to  $> 8 \mu\text{g}/\text{ml}$ ) and chloramphenicol (2 to 4  $\mu\text{g}/\text{ml}$ ). The greatest range of MIC was seen when using erythromycin (1 to  $> 8 \mu/\text{ml}$ ) and tetracycline (0.5 to  $> 8 \mu\text{g}/\text{ml}$ ). The MIC measured for the four control organisms were in accord with published data (Table 2).

## DISCUSSION

In the United States vancomycin is the drug of choice for the treatment of clostridial infections of the human bowel (Finegold and George, 1988). For reasons of its high cost vancomycin is only rarely used to treat similar conditions in other animals; a good alternative is bacitracin. Although *C. spiroforme* was relatively resistant to both compounds (Table 2), the levels of each which can be reached within the bowel are high – neither are well absorbed from the gut – and adequate for antimicrobial activity (Finegold and George, 1988). Even so, the disease in rabbits is such that the time between the first apparent signs (lethargy and perianal soiling by faeces) and the almost inevitable death is frequently less than 8 h (Carman and Borriello, 1988). Consequently, it is by no means certain that intervention with either bacitracin or vancomycin would prove an effective treatment after the onset of clinical signs. However, Katz et al. (1978), were able to prevent the induction of scouring by clindamycin when vancomycin was given simultaneously. If only the former was used, diarrhoea and death soon followed.

Willis (1977) cautioned against the use of both erythromycin and tetracycline for the treatment of uncharacterized anaerobes because of only erratic success. The reasons are that the MIC of these agents show the widest range of the compounds tested and because of the presence of resistant strains.

Despite the uniform sensitivity of all 11 strains of *C. spiroforme* to metronidazole (Table 2), this drug is unlikely to be effective in treating already present disease for the same reason (see above) that bacitracin and vancomycin are probably ineffective. Furthermore, rabbits are reared intensively; sick animals in farm colonies, and to a lesser extent, in research laboratories, are rarely culled or quarantined soon enough to stem the spread of the toxigenic organism to other animals and their environment. In short, antibiotic therapy

is hardly ever applied soon enough or vigorously enough to prevent the rise in environmental contamination and subsequent elevation in the rate of infection. Mass medication, rather than culling or quarantining diseased rabbits, is likely to be the appropriate method for the control of *C. spiroforme* mediated diarrhoea within rabbit colonies; metronidazole would be the choice of many as a suitable compound. However, when dimetridazole and ipronidazole, imidazole compounds with broad spectrum anti-anaerobe activity very similar to that of metronidazole, were added to food and/or water over a period of six months, thus treating all individuals within an infected rabbit colony, the morbidity and mortality resulting from infection with *C. spiroforme* remained unchanged. Despite the lack of success every isolate of *C. spiroforme* cultured from that colony was sensitive to dimetridazole, ipronidazole as well as metronidazole (Carman, 1987). It is not clear whether such an approach would be more successful if applied before the start of an outbreak.

Several antimicrobial agents can induce *C. spiroforme* mediated scours in rabbits; they are penicillin G, ampicillin, amoxicillin, erythromycin, cephalothin, clindamycin (Carman and Evans, 1984) and lincomycin (Rehg and Pakes, 1982). Table 2 shows that two of these compounds, lincomycin (11 strains) and erythromycin (9 strains), had MIC of 8 µg/ml or more. It is appealing to suggest that small numbers of resistant clostridia, resident within the gut, grow into the vacant ecological niche created by antibiotic. However, this is unlikely as Carman and Borriello (1984) showed that, following challenge of healthy rabbits with antibiotic, containment within a positive pressure flexible-film isolator prevented progression to disease. Only when the rabbits were exposed to *C. spiroforme* did diarrhoea ensue. It was proposed that a perturbation of colonization resistance of the normal flora resulted from the use of antibiotics and that the disease was the result of a true infection and not the overgrowth of endogenous bacteria.

Table 2 shows that the test strains were consistently resistant, in vitro at least, to several of the agents studied. Exploiting these resistances, using either single agents or mixtures of two or more, it should be possible to develop a medium for the selective isolation of *C. spiroforme* from faeces or caecal content. We suggest that because of their spectra of activity against other types of bacteria, a combination of rifampicin with either nalidixic acid and/or bacitracin could be a useful adjunct to the selectivity culturing of *C. spiroforme*.

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