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Technical method

Effect of antibiotic concentration in a selective medium on the isolation of *Clostridium difficile* from faecal specimens

PN LEVETT *PHLS Anaerobe Reference Unit, Public Health Laboratory, Luton and Dunstable Hospital, Luton LU4 0DZ*

The presence of *Clostridium difficile* in stools may be inferred by the detection of the toxin(s) produced by the organism or confirmed by isolation of the organism itself. Efficient selective media are now available,[1–3] supplemented by enrichment techniques,[4–7] which increase considerably the sensitivity of cultural methods.

The original egg yolk selective medium (CCFA)[1] contained cycloserine (500 mg/l) and cefoxitin (16 mg/l), but subsequent modifications[2 3] used only half these concentrations of antibiotics. This report presents the results of an investigation of the effect of antibiotic content of CCFA medium on the isolation of *C difficile* from faeces.

Material and methods

Stool specimens submitted to the Anaerobe Reference Unit for examination for *C difficile* were processed on the day of receipt and then stored at 4°C. The initial cultural examination entailed the use of alcohol shock,[8] followed by plating on to blood agar and modified CCFA medium.[3] Stools from which *C difficile* was isolated were re-examined as soon as possible, all within 72 h of receipt.

Thirty three positive specimens were subjected to alcohol shock followed by plating on to blood agar and duplicate plates of modified CCFA medium. This medium was prepared as described previously[9] but contained cycloserine and cefoxitin at concentrations of 250 mg/l and 8 mg/l respectively or 500 mg/l and 16 mg/l respectively. After incubation in an anaerobic cabinet at 37°C for 48 h the degree of growth was estimated on a scale of + to +++++. *C difficile* was identified using the criteria of Holdeman *et al*.[10]

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Results

C difficile was isolated from all of 33 faecal specimens plated on to CCFA containing cycloserine and cefoxitin at concentrations of 250 and 8 mg/l respectively, but from only 25/33 (75%) specimens plated on to CCFA containing double the concentrations of antibiotics (see Table). Moreover, the degree of growth apparent on the plates containing the high concentrations of cycloserine and cefoxitin was invariably inhibited compared with that obtained on the plates containing the lower antibiotic concentrations. There was no difference in the growth of contaminating organisms on plates containing either concentration of antibiotics.

Discussion

The results presented here indicate that the use of cycloserine and cefoxitin in selective media for *C difficile* at concentrations of 250 mg/l and 8 mg/l respectively may effectively increase by 30% the isolation rate of *C difficile* obtained using CCFA medium as originally described,[1] when used in conjunction with an alcohol shock technique. The antibiotic concentrations initially used by George *et al*[1] were derived from a study of the susceptibilities of 14 strains of *C difficile* to 14 antibiotics.[11] The lowest minimum inhibitory concentrations of cycloserine and cefoxitin recorded were 1024 mg/l and 32 mg/l, respectively. In a further study, however, 8% of 39 strains had minimum inhibitory concentrations to cycloserine of 512 mg/l.[12] Moreover, Dzink and Bartlett[13] found 15% of 84 strains to be inhibited by 16 mg/l cefoxitin; similarly, two of 40 strains had minimum inhibitory concentrations to cefoxitin of 16 mg/l or less.[14]

Given the importance of *C difficile* as the causative organism in most cases of antibiotic associated colitis[15–17] and its association with other gastrointestinal tract conditions[18–22] the isolation methods used should be designed so as to yield the maximum possible recovery rate. In this study 8/33 strains of *C difficile* were not recovered from known positive stools using a selective medium containing the higher concentrations of the two antibiotic combination. Direct plating of stools on to modified CCFA medium would not necessarily have given the same results. Nevertheless, the value of alcohol shock as a selective antecedent to plating on to modified CCFA medium has been shown.[6] The use of alcohol shock and a selective medium containing cycloserine and cefoxitin at concentrations of 250 mg/l and 8 mg/l respectively is highly recommended.

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Effect of antibiotic concentration on isolation of Clostridium difficile on egg yolk CCFA after alcohol shock

Specimen	*Cycloserine/cefoxitin concentration (mg/l)* 500/16		250/8	
	C difficile	*contaminants*	*C difficile*	*contaminants*
1	−	+	+++	+
2	−	+	++++	−
3	−	+	++	−
4	+	+	++++	+++
5	+++	−	++++	−
6	+++	+	+	−
7	+	−	+++++	−
8	+	+	+++++	+
9	++	−	++++	−
10	−	−	++	−
11	−	−	+++	−
12	−	+	+++	+
13	+++	−	+++++	−
14	−	−	++	−
15	+	−	++	−
16	++++	++	+++	+
17	++++	+	+	+
18	−	++	++	−
19	+++	+	+++	+
20	++	−	+++	−
21	++++	+	++++	+
22	−	+	++++	++
23	+++	+	+++	+
24	++	+	++	+
25	++	−	+++	−
26	++++	+	+++++	+
27	+++++	+	+++++	+
28	+++++	−	+++++	−
29	++	−	+++	−
30	+++++	+	+++++	+
31	++	+	++++	+
32	++	++	+++++	++
33	+++++	−	+++++	−

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Requests for reprints to: PN Levett, Public Health Laboratory, Luton and Dunstable Hospital, Lewsey Road, Luton LU4 0DZ, England.