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Faecal carriage of *Clostridium perfringens*

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SUMMARY

The numbers and serotypes of *Clostridium perfringens* present in the faeces of three groups of hospital patients and young healthy laboratory workers were examined in studies lasting between 10 and 13 weeks.

In one hospital some long-stay geriatric patients carried relatively high numbers of *C. perfringens* ($>10^7$/g) most of the time and it was not unusual in any one week for the majority of these patients to carry the same serotype(s). However, the numbers of *C. perfringens* in the faeces of young long-stay patients in the same hospital were in the range of 10^3–10^4/g and carriage of common serotypes was not observed. These results were similar to the findings with the young laboratory workers.

This investigation indicates that two of the laboratory criteria often used in the investigation of *C. perfringens* food poisoning, i.e. faecal counts of $\geqslant 10^5$ *C. perfringens*/g and patients carrying the same serological type need to be interpreted with caution with suspected outbreaks involving some groups of geriatric long-stay hospital patients.

INTRODUCTION

Clostridium perfringens is recognized throughout the world as an important cause of food poisoning (Stringer, Turnbull & Gilbert, 1980). Until the reports by Hall *et al.* (1963) and Taylor & Coetzee (1966), great emphasis was placed on the isolation of 'heat-resistant' strains of *C. perfringens* (i.e. those strains whose spores were capable of surviving heat treatment at 100 °C for 1 h). However, it is now widely accepted that 'heat-sensitive' strains are equally capable of causing food poisoning (Sutton & Hobbs, 1968).

C. perfringens is not a major component of the human intestinal flora when compared to the level of strict anaerobes which may be isolated when suitable techniques are employed, but it can be isolated with relative ease from nearly all

faecal specimens. Faecal carriage of *C. perfringens* has been studied primarily in relation to its diagnostic significance in the investigation of suspected outbreaks of food-borne disease, and it was soon realized that it was important to estimate the numbers of *C. perfringens* in faecal specimens. Sutton (1966) showed that healthy people usually carried between 10^3 and 10^4 organisms/g whereas patients in outbreaks tended to carry 10^5–10^6/g. In 1976, Yamagishi *et al.* found that the numbers of *C. perfringens* in the faeces of some healthy adults in a Japanese Home for the Aged were consistently of the order of 10^7–10^9/g.

During the investigation of food poisoning outbreaks in hospitals in the United Kingdom, the Food Hygiene Laboratory has often encountered high levels (greater than 10^7/g) of *C. perfringens* in the faeces of patients considered to be control subjects. It was also observed that, on occasions, some serotypes were unusually common in certain institutions. During 1977–9 five outbreaks of *C. perfringens* food poisoning occurred in a hospital and serotype 28 was isolated in large numbers from patients on all occasions. In the same period there was a similar series of ten incidents in another hospital and serotype 41 was suspected as the causative organism of four of the outbreaks whilst some other serotypes were isolated repeatedly from faecal specimens associated with a number of these ten episodes (Stringer, 1981).

In view of these findings and the results of the Japanese study, it was decided to examine the numbers and serotypes of *C. perfringens* carried by long-stay hospital patients and healthy laboratory staff.

MATERIALS AND METHODS

Details of patient groups

Group A. This comprised 11 long-stay geriatric patients in a general hospital, average age 77 years (range 65–92), who had been in hospital for at least 3 months prior to the study. The principle diagnoses were cerebral vascular accident (7), congestive heart failure (2), osteoarthritis and leg amputation (1 each).

Group B. Six young patients in the same general hospital as the Group A patients. Four had been hospitalized for at least 1 year and were suffering from multiple sclerosis (2), traumatic quadriplegia and cerebral palsy (1 each). The other two patients had been admitted 1 month before the study with fractured femurs. The average age was 27 years (range 16–39).

None of the patients in Group A and B was known to have disease of the gastrointestinal tract and all took a normal diet. None had received antibiotic for at least 2 weeks before commencement of the study. The investigations of the Group A and B patients were not concurrent, the second commenced 2 months after completion of the first.

Group C. Ten elderly patients in a hospital for the mentally handicapped who were not suffering from any other medical condition. The average age was 74 years (range 53–85).

Group D. Seven young laboratory staff working in the same institute. The average age was 31 (range 26–41). Four of the workers ate their midday meal in the laboratory canteen (usually the same meal).

Collection of faecal specimens

Faecal specimens were collected from the group A patients on the Monday of 13 consecutive weeks or on the earliest possible day thereafter. Some of these patients regularly took mild laxatives, but deliberate purging to obtain specimens was not permitted. Specimens were transported within a few hours to the examining laboratory. Specimens were collected from group B, C and D subjects in a similar manner but for periods of 12, 10 and 12 weeks respectively.

Collection of food samples

During the period of collection of faecal specimens from the Group A patients a sample of the preceding Sunday's lunch was also examined. This was kept in the deep-freeze in the hospital kitchen and collected for examination on the following day. There was no choice of menu for Sunday lunch and each sample meal therefore consisted of a single meat course with vegetables and a sweet.

Enumeration procedures for faecal specimens from group A and B patients

C. perfringens *total viable count*. A portion of the faecal specimen of approximately 1 g was weighed into a 1 oz screw-capped bottle containing glass beads and the appropriate amount of Ringer solution added to make a 1-in-10 dilution. After shaking to homogenize the bottle was left for a few minutes to allow the debris to sediment. The supernatant was inoculated on to blood agar containing 100 mg/ml neomycin using a Spiral Plater (Don Whitley Scientific, Shipley, Yorkshire) according to Gilchrist *et al.* (1973). For specimens in which a high count was expected a dilution of 1 in 10^3 was also plated using the Spiral Plater.

After overnight incubation of the plate at 37 °C in an anaerobic jar, discrete colonies were counted on an area of the plate showing suitable density of growth. Counting was performed over a grid previously calibrated by the use of suspensions of known viable count, to relate area of plate to volume of sample. This method could detect 5×10^2 *C. perfringens* per gram of faeces, but 5×10^3 was regarded as the lower limit of an accurate count.

C. perfringens *spore count*. The 1-in-10 and, if appropriate, the 1-in-1000 dilutions prepared for the total viable count were heated at 80 °C for 10 min and the spiral plate counts repeated.

Enumeration procedures for faecal specimens from group C and D patients

C. perfringens *total viable count*. One gram of each faecal specimen was added to 9 ml of Ringer solution and tenfold dilutions were prepared in further 9 ml volumes of the same solution up to 1 in 10^6. Colony counts on blood agar containing neomycin (100 mg/ml) were carried out using a modification of the surface-drop technique of Miles & Misra (1938) as described by Thatcher & Clark (1968). Plates were incubated anaerobically at 37 °C for 24 h.

C. perfringens *spore count*. The 1-in-10 dilution of faeces prepared for the total viable count was heated at 80 °C for 10 min and the count procedure repeated to obtain the spore count.

Isolation of heat-resistant strains

A small portion of the faecal specimen was added to a cooked-meat broth which was heated at 100 °C for 1 h, cooled under tap water and incubated at 37 °C for 18–24 h. The cooked-meat culture was then plated on to neomycin blood agar that was incubated anaerobically at 37 °C for 24 h. The presence of growth indicated that the *C. perfringens* were of the heat-resistant type.

Confirmatory tests

Representative isolates from group A and B patients were subcultured from neomycin blood agar on to plates of neutral-red/lactose/egg-yolk medium (Willis & Hobbs, 1959) of which half the plate had been spread with *C. perfringens* type A antitoxin (Wellcome Laboratories, Beckenham, Kent). After anaerobic incubation for 18–24 h at 37 °C strains which showed specific inhibition by the antitoxin and fermented lactose were considered to be *C. perfringens* type A. Isolations from group C and D patients were confirmed as *C. perfringens* using the Nagler reaction on nutrient egg-yolk medium according to McClung & Toabe (1947).

All strains that were subsequently found to be serologically non-typable were further confirmed as *C. perfringens* by a short series of biochemical tests. Actively growing cultures in cooked-meat broth were inoculated into glucose, lactose, sucrose, salicin and raffinose peptone waters, gelatin agar, litmus milk and two peptone waters (for the detection of indole and H_2S production). All tests were incubated at 37 °C for 24–72 h and carried out and interpreted according to Cowan & Steele (1965). The isolates were also tested for motility and the ability to reduce nitrate according to the method of Hauschild & Hilsheimer (1974).

Serological typing

Serotyping of isolates of *C. perfringens* was carried out by slide agglutination with the 143 antisera used routinely at the Food Hygiene Laboratory in the investigation of outbreaks of food poisoning (Stringer, Turnbull & Gilbert, 1980).

Preparation of a new serum against a non-typable strain

Towards the end of the survey of the group A patients a high proportion of the faecal isolates were serologically non-typable. In an attempt to determine whether the strains were the same type, a new serum was prepared against a non-typable strain isolated from patient K in week 12. The antiserum was prepared according to the method of Hughes, Turnbull & Stringer (1976). The new serotype was designated type 90.

RESULTS

Counts of Clostridium perfringens *in faeces*

In general, with all four groups of subjects studied the spore counts correlated well with the total viable counts. Although the spore counts were usually two-thirds that of the total viable count, occasionally when very low numbers were recorded spores were not detected and, conversely, there were a few instances when the total

Table 1. *Total viable counts of* Clostridium perfringens *in the faeces of long-stay geriatric patients (group A), long-stay young adult patients (group B), mentally handicapped patients (group C) and young healthy laboratory workers (group D)*

	Patient code	Week number and *C. perfringens* count*													Median* count
		1	2	3	4	5	6	7	8	9	10	11	12	13	
Group A	A	8·6	6·8	6·5	6·0	5·4	7·1	8·7	8·9	8·8	8·8	8·8	9·0	8·8	8·7
	B	5·3	5·0	—	—	—	5·7	—	7·0	8·6	—	8·6	—	8·9	7·0
	C	8·2	5·8	6·0	7·7	8·0	8·6	8·6	8·6	8·7	8·2	8·7	7·9	—	8·2
	D	5·9	4·7	5·4	8·8	9·0	—	5·6	6·0	6·7	—	—	—	8·8	6·0
	E	5·0	5·0	P	—	P	—	4·5	—	—	—	—	—	4·0	4·5
	I	—	—	7·2	8·6	8·5	8·8	9·2	10·0	10·2	9·0	9·2	9·0	9·6	9·0
	J	6·4	7·3	6·4	7·1	5·2	5·2	5·9	5·8	5·7	5·9	5·7	6·2	6·5	5·9
	K	5·4	5·1	P	ND	ND	ND	—	5·4	5·9	5·3	5·3	5·8	6·6	5·4
	L	4·3	—	P	—	P	—	—	—	—	4·0	—	—	—	4·0
	N	ND	ND	P	ND	ND	ND	P	4·0	P	P	ND	4·0	P	4·0
	P	P	P	P	ND	ND	ND	P	—	4·1	5·6	—	5·1	P	<3·7
Group B	Q	4·3	—	—	4·8	5·1	4·4	3·9	3·8	—	3·9	3·8	3·9		4·3
	S	3·8	4·3	4·8	P	4·4	P	3·8	4·7	4·4	5·5	6·9	4·8		4·4
	T	P	ND	P	ND	ND	P	P	—	P	P	—	—		<3·7
	U	P	—	P	ND	—	—	—	—	—	—	—	—		<3·7
	V	—	—	—	—	P	ND	—	—	—	—	—	—		<3·7
	W	—	—	—	—	ND	ND	4·2	—	4·7	ND	—	—		4·0
Group C	MO	6·7	3·9	5·3	4·5	4·5	ND	7·3	ND	P	4·5				4·5
	WE	3·8	3·8	ND	P	ND	ND	ND	5·8	ND	8·3				<3·0
	BA	ND	ND	ND	4·9	ND	ND	—	ND	ND	ND				<3·0
	NI	6·6	8·0	7·2	6·7	6·8	8·1	8·4	7·8	7·0	8·4				7·8
	CL	ND	ND	4·7	P	4·2	P	4·8	ND	5·3	P				<3·0
	RO	5·4	ND	4·1	P	6·9	ND	3·9	8·2	8·2	ND				4·1
	WI	4·1	6·4	6·4	P	5·2	P	6·1	6·1	6·4	6·5				6·1
	GR	7·6	7·7	7·5	6·2	7·6	8·1	8·1	7·2	8·1	7·8				7·7
	BR	ND	ND	ND	ND	ND	ND	ND	—	—	—				<3·0
	BL	5·8	ND	—	—	4·4	ND	—	—	—	—				4·4
Group D	MS	ND	ND	2·7	3·8	ND	ND	3·4	5·5	4·7	5·9	4·8	ND		3·4
	JS	ND	ND	ND	ND	ND	ND	7·0	4·7	ND	ND	ND	ND		<2·7
	DR	5·9	4·0	3·2	5·3	3·7	6·9	5·8	5·8	6·6	5·5	6·9	2·9		5·8
	JK	2·7	2·7	ND	ND	ND	ND	3·4	3·9	2·7	ND	ND	ND		<2·7
	AG	5·9	4·4	3·3	ND	3·2	5·7	ND	3·0	4·0	2·9	ND	ND		3·2
	NS	6·2	6·9	ND	3·5	ND	4·5	6·3	5·5	3·5	4·1	2·9	ND		4·4
	JP	3·9	5·0	4·4	ND	4·1	5·2	5·2	5·0	4·3	ND	4·3	4·3		4·3

P, Present but $<5{\cdot}0 \times 10^3$ organisms/g. ND, Not detected. —, Faecal specimen not obtained.
* Log_{10}/g.

viable count was $<2 \times 10^2$/g and small numbers of spores were present. The comparative results presented in Table 1 are the total viable counts for all four groups of patients.

Group A. Between 4 and 13 faecal specimens were collected from the geriatric patients over the course of 13 weeks. During this period some of these patients carried relatively high numbers of *C. perfringens* most of the time (i.e. patients A, C and I), whereas other patients (E, K, L and P) carried low numbers. Three patients (B, D and J) had median counts between the 'high' and 'low' carriage

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Table 2. *Predominant serotypes of* Clostridium perfringens *isolated from the faeces of long-stay geriatric (group A) and mentally handicapped (group C) hospital patients*

	Patient code	No. of specimens*	Predominant serotypes (number of weeks)
Group A	A	13	25/34/68 (10), 50/74 (3), 90 (3)
	B	7	25/34/68 (2), 32 (3),
	C	12	25/34/68 (10), PS.18 (3), 90 (2), 33/61 (3)
	D	9	25/34/68 (4), 41 (5),
	E	6	PS.74 (2), 41 (2),
	I	11	25/34/68 (3), 32 (4), 90 (4)
	J	13	33/61 (6), PS.18 (5), 90 (3)
	K	9	25/34/68 (3), 32 (3), 90 (3)
	L	4	41 (2)
	N	7	28 (4)
	P	8	38 (3), 33/61 (2)
Group C	MO	8	21 (2)
	WE	5	
	BA	1	
	NI	10	36 (5), 10 (3), 7 (2)
	CL	7	41 (4)
	RO	7	34 (3)
	WI	10	28 (2), 52 (2)
	GR	10	28 (10)
	BR	0	
	BL	2	

* Number of faecal specimens from which strains of *C. perfringens* were obtained for serological typing.

Table 3. *Predominant serotypes of* Clostridium perfringens *isolated each week from the faeces of long-stay geriatric patients (group A)*

Week no.	No. of specimens*	Predominant serotypes (number of patients)
1	9	25/34/68 (7), 33/61 (3)
2	8	25/34/68 (3), 33/61 (5), TW.23 (3)
3	10	25/34/68 (4), 33/61 (2), 41 (2)
4	5	25/34/68 (3), 33/61 (2)
5	6	25/34/68 (3)
6	5	25/34/68 (3), 33/61 (2)
7	8	25/34/68 (2), 90 (2)
8	8	32 (3), 25/34/68 (2), PS.18 (2)
9	9	32 (6), 25/34/68 (2), PS.18 (2), 28 (2)
10	9	32 (6), 25/34/68 (2)
11	5	90 (5)
12	7	90 (5), 25/34/68 (2)
13	9	90 (6), 25/34/68 (2)

* See footnote on Table 2.

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Table 4. *Predominant serotypes of* Clostridium perfringens *isolated each week from the faeces of mentally handicapped patients (group C)*

Week no.	No. of specimens*	Predominant serotypes (no. of patients)
1	7	28 (2), 68 (2)
2	5	
3	7	21 (2)
4	8	28 (3)
5	7	
6	4	
7	6	28 (2), 7 (2)
8	5	
9	6	28 (2)
10	6	41 (2)

* See footnote on Table 2.

Table 5. *Summary of serological typing results on strains of* Clostridium perfringens *isolated from hospital patients and laboratory staff*

Patient group	No. of faecal specimens		No. of different serotypes	No. of faecal specimens from which non-typable strains were isolated
	Examined	Positive for *C. perfringens*		
A	111	98 (88)	29	16 (16)
B	40	32 (80)	29	21 (66)
C	90	52 (58)	22	20 (38)
D	84	53 (63)	43	47 (89)

Percentages in parentheses.

patients, although in some weeks high counts (10^7/g) were obtained from all three of these patients. Forty-six (55%) of the 84 faecal specimens from which counts of *C. perfringens* were made contained $\geqslant 10^6$ organisms/g and 31 (37%) of these had 10^8 organisms/g. With two of the patients (C and J) there was evidence to suggest a steady change in the numbers of *C. perfringens* carried but other patients showed a more erratic carriage pattern.

Group B. It proved difficult to obtain regular faecal specimens from the young patients in the same hospital and during the 12-week investigation only three of the six patients provided more than five specimens. Greater than 10^5 organisms/g were present in only 3 (14·3%) of the 21 specimens that were positive for *C. perfringens.*

Group C. The mentally handicapped patients were studied for 10 weeks and during this time 9 of the 10 patients provided seven or more specimens. Two of these patients (NI and GR) carried relatively high numbers most of the time (i.e. $> 10^7$/g). Six of the 10 counts from patient WI were of the order of 10^6/g and the average counts for a further six patients were in the range of 10^3–10^4/g. Thirty-two (62%) of the 52 faecal specimens from which *C. perfringens* was isolated contained $> 10^6$ organisms/g and 20 (38%) of these had $> 10^7$/g.

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Group D. Counts from six of the young healthy laboratory workers were usually between 10^3 and 10^4/g and the remaining volunteer had a median count of 10^5/g during a 12-week period of investigation. Individual JS was unusual since in 10 of the 12 weeks *C. perfringens* was not isolated; however, prior to the collection of the specimen in week 7, JS suffered typical symptoms of *C. perfringens* food poisoning 12 h after the consumption of a chicken meal. This illness was probably responsible for the very high faecal count of *C. perfringens* obtained in week 7 – the highest count found in any of the group D subjects during the 12-week survey. By week 8 the count had dropped to 10^4/g and *C. perfringens* was not found in the faeces of JS during the remainder of the survey. Only 7 (13 %) of the 53 faecal specimens collected from the group D subjects contained *C. perfringens* in excess of 10^6/g.

There was no evidence of a relationship between age and the presence of high or low numbers of *C. perfringens* in patients belonging to any group.

Carriage of C. perfringens *serotypes*

The serotypes of *C. perfringens* isolated from group A and group C patients are shown in Table 2. For each patient the predominant types (i.e. those isolated on two or more occasions) are recorded and in parentheses the number of weeks they were isolated.

Group A. It was evident that some patients could carry the same type for a long period of time, e.g. type 25/34/68 was isolated from patient A in 10 of the 13 weeks. This same type was also isolated from patient C in 10 of the 12 weeks investigated. Other types were more prevalent in other patients, e.g. type 33/61 was recovered from the faeces of patient J on 6 weeks, type PS.18 on 5 weeks and serotype 41 was found in 5 of the 9 faecal specimens collected from patient D. The new type 90 strain was isolated on 2 or more weeks from 5 of the patients.

Group B. Owing to the relatively small numbers of specimens collected from these patients it was not possible to observe carriage of particular types. However, serotype 33 was isolated from patient Q on 3 of 9 weeks in which a specimen was positive for *C. perfringens.*

Group C. Serotypes 36 and 10 were carried by patient NI for 5 and 3 weeks respectively, type 41 by patient CL for 4 of 7 weeks and patient GR carried type 28 continuously throughout the 10-week investigation.

Group D. Only two individuals carried a type for more than 2 of the 12 weeks. Type 31 was isolated from patient AG on weeks 1, 5 and 9 and type 28 from patient JP on weeks 1, 7 and 12.

The predominant serotypes of *C. perfringens* isolated each week from group A and C patients are shown in Tables 3 and 4 respectively. Some serotypes were unusually common; for example, in week 1, type 25/34/68 was isolated from 7 of the 9 geriatric patients examined. This type, along with 33/61, was isolated from 2 or more patients during weeks 1–4. In week 5, type 25/34/68 was isolated from 3 of the 6 specimens examined. For the purposes of this investigation a serotype was considered significantly common if it was isolated from 30 % or more of the patients examined in any 1 week. Following this criterion, type 25/34/68 was a common type in weeks 1–6, in week 7 there was no predominant type, but type 90 was isolated for the first time. During weeks 8–10, type 32 was common, and

in weeks 11–13, type 90 established itself as the prevalent type. In fact, type 90 was found in 16 (76%) of the 21 faecal specimens from which *C. perfringens* was isolated during weeks 11–13. With the young hospital patients, in only 1 week did two patients carry the same type. The serotypes frequently isolated from the geriatric patients (i.e. 25/34/68, 33/61, PS.18 and type 90) were not found in the faeces of young patients in the same hospital. Serotype 28 was isolated from two or more group C patients on 4 of the 10 weeks and in only 2 of the 12 weeks did two or more group D subjects yield the same serotype.

A summary of the serological typing results on the four groups of subjects is presented in Table 5. A comparison of the ratio of the number of specimens positive for *C. perfringens* to the number of different serological types for each group clearly shows that a much wider range of serotypes was isolated from the young subjects (groups B and D) than from the elderly patients (groups A and C). Also, non-typable strains were isolated far more frequently from the young subjects than the older hospital patients. Only 16 (16%) of the 111 faecal specimens from the geriatric patients (group A) yielded non-typable isolates; however, this figure would have been higher if a new serum had not been prepared against one of the isolates from a geriatric patient.

Usually, strains isolated from unheated specimens (total viable cell count) and heated specimens (spore count) were subsequently shown to be the same serotype. However, occasionally it was possible to isolate a serotype from the heated specimen instead of, or in addition to, the serotype(s) isolated from the unheated specimens. There was no evidence of any exclusive relationship between the carriage of high numbers of *C. perfringens* and particular serotypes. There was also no correlation between the carriage of serotypes and the wards in which the hospital patients were situated. During the survey of group A patients, *C. perfringens* was isolated from only one sample of food; a non-typable strain was isolated on enrichment in week 12.

DISCUSSION

In the investigation and confirmation of *C. perfringens* food poisoning one or more of the following laboratory criteria are usually applied: (*a*) the isolation of organisms of the same serotype from the epidemiologically incriminated food and the faeces of ill persons, (*b*) the isolation of organisms of the same serotype from the faeces of most of the ill persons but not from the faeces of controls, (*c*) the isolation of 10^5 organisms per gram from the epidemiologically incriminated food and (*d*) a faecal spore count of 10^6/g in most of the ill persons examined. The results presented in this investigation of the carriage of high numbers of *C. perfringens* (i.e. $>10^7$/g) by some of the group A patients, together with the demonstration that in any 1 week several of these patients could carry the same serotype (e.g. 7 of 9 patients carried type 25/34/68 in week 1 and all 5 patients sampled in week 11 yielded type 90) indicate a severe limitation in the application of the above criteria. During the course of this survey none of the group A patients suffered from any form of gastroenteritis or diarrhoeal illness and the high numbers of *C. perfringens* carried by these patients was probably a reflexion of the normal

carriage situation for some elderly institutionalized or hospitalized patients. These results therefore support the findings of Yamagishi *et al.* (1971) and Sutton (1966). If a cluster of cases of diarrhoea occur in such patients it is not unreasonable, following the laboratory examination of faecal specimens, to assume that *C. perfringens* food poisoning is responsible. Therefore to clarify such a situation it is most important that the date of onset of illness is recorded, symptoms are accurately described and a food-attack rate analysis carried out to obtain a complete epidemiological record of the suspect incident. Experience has shown that this approach is essential to enable the interpretation of serological typing results of questionable significance.

Not all elderly persons in hospitals or institutions will carry high numbers of *C. perfringens*, as this study has shown, with some of the group A patients and with most of the group C patients situated in a different hospital. However, it was of interest to record that the young long-stay patients (group B) in the same hospital as the group A patients did not carry high numbers of similar serotypes and that the carriage pattern in group B was similar to that of the healthy laboratory workers (group D).

The hospital in which the group A and B patients resided had not been the subject of previous investigations for *C. perfringens* food poisoning, but this was not the case with the hospital in which the group C patients were situated. In 1980 there had been a confirmed outbreak of *C. perfringens* food poisoning in which serotype 52 was isolated in large numbers from 11 of 12 patients investigated. A year later there was a second 'suspected' outbreak of food poisoning in the same hospital and type 52 was isolated from 9 of 10 patients examined. On this occasion food was not shown to be the source of infection and symptoms were experienced by patients over the course of several days. During March and April of 1981 a 'follow-up' survey was conducted and 5 patients involved in the 1980 outbreak were examined. Although type 52 was isolated from all of them at the time of the outbreak it was not isolated during the follow-up. In contrast, serotype 52 was isolated from 5 of 8 patients previously involved in the 1981 incident. In addition, serotype 52 was also isolated from the 3 patients unrelated to the 1980 or 1981 incidents. It was these events which led to the survey of the group C patients. Immediately following the survey reported here, faecal specimens were collected from 18 other patients in the same hospital but not in group C, and serotype 52 was isolated from 8 of them. One of these patients was ill in the 1981 incident and was also sampled in the follow-up survey, and on both of these previous occasions serotype 52 was also isolated. Although type 52 was undoubtedly present in the faeces of many of the patients in this hospital at different times, it was only isolated on three occasions (two patients) during the course of the present study. Only one of the group C patients had been involved in the 1980 or 1981 incidents.

In a study of 890 strains of *C. perfringens* isolated from faeces during the investigation of 587 outbreaks of *C. perfringens* food poisoning and shown not to be the outbreak-causing type, only eight of the strains belonged to type 52 (Stringer, 1981). This same type was identified as the causative organism in only 3 of the 587 outbreaks. It is therefore evident that serotype 52 was unusually prevalent in this hospital for a long period of time. Apart from type 41, the serotypes frequently isolated from the group A patients (i.e. 25/34/68, 33/61, 32

and PS.18) were not a common cause of *C. perfringens* food poisoning during 1970–9 and they were isolated infrequently from faeces. Type 28, which was isolated from a number of group C patients, is not frequently associated with food poisoning but is considered to be one of the most common 'normal flora' types. Surprisingly, the most common type isolated from the group A patients (type 25/34/68) had never been previously isolated.

A wide range of factors influence the bacterial flora of the intestine and Drasar & Hill (1974) grouped them under three headings: (*a*) host physiology, (*b*) environmental factors and (*c*) bacterial interactions. Factors such as intestinal secretions, intestinal mucosa, immune mechanisms and intestinal motility may be important influences on the presence and stability of *C. perfringens* in the intestine. It is unlikely that consumption of common food is responsible for the carriage of the same serotypes, since *C. perfringens* was only isolated from one of the meals examined in this investigation. Of the environmental factors, it is possible that bacterial contamination and transmission by a variety of routes enable some serotypes to establish themselves in certain communities. In a study of the epidemiology of enterotoxigenic *C. perfringens* as a cause of antibiotic-associated diarrhoea Borriello *et al.* (1984) described a cluster of five cases of diarrhoea due to serotype 41, which was closely followed by a further three and two cases due to serotypes 27 and 24 respectively. The isolation of these serotypes of *C. perfringens* from various patient groups, nursing staff and environmental samples suggested that *C. perfringens* may act as a true infectious agent.

The strains of *C. perfringens* isolated during this investigation were not examined for their ability to produce enterotoxin *in vitro*. However, Stringer, Watson & Gilbert (1982) reported that enterotoxigenicity in strains other than those associated with food poisoning is uncommon. With the advent of improved techniques for sporulation and the development of a highly sensitive and rapid technique (ELISA) for the detection of enterotoxin (Bartholomew *et al.* 1985) an investigation of the potential for toxin production by normal flora isolates would be worthwhile.

In summary, it is evident that the carriage of *C. perfringens* varies considerably between individuals and that in some hospitals and old peoples' homes elderly patients may carry high numbers of the same serotypes. It is therefore recommended that the investigation of outbreaks in such places should include an extensive epidemiological investigation. More recently, Bartholomew *et al.* (1985) have shown that the detection of *C. perfringens* enterotoxin in faecal specimens obtained within two days of the onset of symptoms typical of *C. perfringens* food poisoning is a specific and significant finding. It is suggested that where possible the presence of enterotoxin in faeces be considered as an important additional criterion to those previously mentioned.

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