Bacteriocin Typing of *Clostridium perfringens* in Human Feces

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Three hundred and ninety-nine isolates of *Clostridium perfringens* from enriched stool specimens of 51 individuals (about eight colonies per person) were typed by bacteriocins. Forty-nine percent of these persons carried more than one bacteriocin type in their stool, and some had three or four different strains as determined by bacteriocin typing. Weekly stool specimens obtained from seven positive volunteers over a period of 5 weeks were screened for *C. perfringens*, and several colonies from each person were typed. This survey demonstrated that the number of types fluctuated with time, several types could be carried simultaneously, and the isolation of the organism was variable. Nine new bacteriocin types of *C. perfringens* were isolated in this study.

In 1974, we (11) proposed a typing scheme for *Clostridium perfringens* using 10 bacteriocins. With this system, 274 strains were divisible into 50 different typing patterns. Many of the bacteria typed at that time were not of human fecal origin, and no information was gathered to evaluate multiple-strain carriage in humans. In this study, we have examined several colonies grown from each individual specimen of human feces to estimate the number of bacteriocin types carried at any one time by any one individual. In addition, weekly fecal samples were collected and studied from some individuals to evaluate the permanence and/or variability of strain carriage.

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Stool specimens delivered to the diagnostic laboratory of the Victoria General Hospital, Halifax, Nova Scotia, served as the major source of the strains of *C. perfringens*. Weekly specimens were obtained from seven departmental volunteers.

Approximately 1 g of each stool specimen was inoculated into 25 ml of cooked-meat broth (BBL), which had been boiled for 10 to 15 min to drive off dissolved oxygen. The specimens were incubated overnight at 37°C and subcultured to two blood agar plates containing 100 µg of neomycin sulfate per ml; these were incubated anaerobically using the GasPak method (BBL). The following day 10 colonies resembling *C. perfringens* were selected from these plates, using colonial morphology and target hemolysis

as aids, and streaked onto blood agar plates for further isolation and testing. The cultures were tested for their ability to ferment dextrose, maltose, lactose, and sucrose, reduce nitrate, liquefy gelatin, and cause stormy fermentation of litmus milk.

After identification was completed, the organisms were stored at 4°C in 10 ml of cooked-meat broth for subsequent bacteriocin typing (maximum holding time, 2 to 3 weeks).

The bacteriocins used in the typing procedure and their preparation have been described elsewhere (11). The organisms to be typed were grown overnight in cooked-meat medium. One milliliter of this medium was then inoculated into freshly boiled and cooled brain heart infusion broth (Difco) and incubated at 37°C for 3 h. The culture was diluted 1:100 in brain heart infusion broth, and this was swabbed onto a blood agar plate (brain heart infusion base; 10% human blood). Ten microliters of each of the 10 bacteriocins (no. 4, 28, 43, 48, 55, 63, 73, 75, 78, and 96) was spotted onto each plate, using a Pipetman P20 pipette. The plates were incubated in an atmosphere of nitrogen at 37°C, and the patterns of inhibition by the bacteriocins were recorded for each strain on the following day. Bacteriocin typing of the cultures was repeated at a later time, and the results were found to be reproducible.

The bacteriocin typing patterns previously proposed by Mahony (11) were revised to include 13 new types, 9 of which were isolated in this study (Table 1). The four other new types were detected in another study of hospital isolates of *C. perfringens*.

Fecal specimens were obtained from 92 individuals. Of these, *C. perfringens* was isolated from 51 specimens. Bacteriocin typing was done on each of 399 isolates (an average of eight colonies per positive culture). All colonies were typable, and almost half (49%) of the stool cultures tested contained more than one bacteriocin type of *C. perfringens*; 33% contained two types; 14% contained three types; and one individual carried as many as four bacteriocin types. Of the 51 individuals, 19 (37%) carried only bacteriocin type C3; 21 (41%) carried type C3 plus other types, and 11 (22%) carried types other than C3. In total, 78% of the people tested carried bacteriocin type C3. Since over half of the colonies

tested were bacteriocin type C3, an attempt was made to divide the group into smaller subgroups by using five additional new bacteriocins (no. 115, 123, 297, 302, and 305) in combination with the ten standard bacteriocins. These 15 bacteriocins were used to type approximately 50% of the total strains tested; however, the additional bacteriocins would not further subdivide any of the existing groups. The C3 strains were consistently sensitive to all of the five additional bacteriocins.

A sequential study was carried out on seven volunteers composed of four males and three females. Six of the individuals provided weekly stool specimens for a total of 5 weeks, and one

TABLE 1. *Thirteen new bacteriocin-typing patterns of C. perfringens*^a

Type	Sensitivity to bacteriocin from strain:									
	4	28	43	48	55	63	73	75	78	96
C12	+	+	—	—	—	—	+	+	+	—
C13	+	+	—	—	+	—	+	+	+	+
D9	+	—	—	—	+	+	+	+	+	+
D10	+	—	—	—	—	—	+	+	+	—
D11	+	—	+	+	+	—	+	+	+	—
D12	+	—	—	—	—	+	+	+	+	+
D13	+	—	—	—	—	—	+	+	+	+
D14	+	—	—	—	+	—	—	+	+	—
D15	+	—	+	—	—	—	+	+	+	—
D16	+	—	—	—	+	+	—	+	+	+
E16	—	+	+	+	+	+	+	+	+	+
G3	—	—	—	—	—	—	—	+	+	—
G4	—	—	—	—	+	+	+	+	+	—

^a See reference 11 for original scheme. +, Susceptible to bacteriocin; —, resistant to bacteriocin.

TABLE 2. *Typing patterns of C. perfringens isolated from weekly stool specimens from seven individuals over a 5-week period*

Individual	Bacteriocin type (no. of colonies)				
	Week 1	Week 2	Week 3	Week 4	Week 5
1	C3(10)	C7(3) D5(7)	C3(10)	C3(10)	A1(1) B1(2) C3(7)
2	C3(10)	C3(10)	C3(6) D10(4)	No specimen	No specimen
3	C3(10)	C3(10)	C3(5)	C3(10)	C3(10)
4	A1(2) C3(5) C7(1)	C3(4) C7(6)	C3(8)	C3(10)	A1(1) C3(7) D10(2)
5	C3(7) C13(2)	C3(1) C7(9)	Negative ^a	C3(6)	Negative
6	D14(10)	Negative	D15(10)	D10(10)	Negative
7	C3(10)	C3(10)	C3(10)	A1(4) C3(6)	C3(10)

^a Negative, *C. perfringens* not isolated.

provided specimens for a total of 3 weeks. The results of this study are presented in Table 2. Two individuals carried a total of four and five bacteriocin types, respectively, over the course of the experiment and as many as three types in any one stool specimen. Also notable in Table 2 is the dominance of bacteriocin type C3. Occasionally, persons carrying *C. perfringens* would have a negative stool specimen, only to be positive again in subsequent sampling. Such data would obviously influence an estimate of the carrier rate in a population where only one sample was examined per individual. This observation may reflect sampling error (1 g of feces may not be microbiologically representative of the total stool), the degree of sporulation of the organism (cultures were not heat shocked), or, less likely, a true loss of flora followed by recolonization. The literature cites much variation in the carrier rate of *C. perfringens* (1-4, 7-10, 12-14). Although the bacteriocin types and proportions of types seemed to fluctuate from week to week in most of the volunteers, one person consistently carried only the C3 bacteriocin type over the 5-week period.

The results of these experiments confirm the serological evidence of others (5, 14) that more than one strain of *C. perfringens* can be carried simultaneously in human feces. The predominance of bacteriocin type C3 in these specimens is obvious, as is the necessity of typing several colonies for any epidemiological study. Further division of the C3 type is required, either through the use of bacteriocins or serology, to determine whether this group of organisms is homogeneous. Subdivision of this group would greatly improve the sensitivity of bacteriocin typing of *C. perfringens*.

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