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Plasmid Analysis as a Means of Strain Differentiation in *Clostridium perfringens*

D. E. MAHONY,[1]* M. F. STRINGER,[2]† S. P. BORRIELLO,[3] AND J. A. MADER[1]

Department of Microbiology, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada,[1] and Food Hygiene Laboratory, Central Public Health Laboratory, Colindale, London NW9 5HT,[2] and Division of Communicable Diseases, Clinical Research Centre, Harrow, Middlesex HA1 3UJ,[3] United Kingdom

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A total of 114 *Clostridium perfringens* isolates were serotyped and examined for plasmids. Fifty-two strains were from hospitalized patients with diarrhea or from hospital environments, and 62 epidemiologically unrelated isolates were obtained from food poisoning outbreaks. All strains were screened for bacteriocin production against a common indicator strain of *C. perfringens*. In the one significant hospital outbreak of *C. perfringens* diarrhea, three to five plasmid types were found in strains of the predominant serotype, but no similar correlation between serotype and plasmid type was found in random isolates from a variety of sources. All of the strains associated with the diarrhea outbreak produced bacteriocins, whereas 63% of the strains from various sources produced bacteriocins. The typing data suggest a promising differentiating capability for plasmid analysis in the epidemiological study of outbreaks of food poisoning, diarrhea, or infections caused by *C. perfringens*.

Clostridium perfringens is a gram-positive, spore-forming, anaerobic bacillus which is found in the soil and intestinal tract as normal flora. In addition, the organism can cause gas gangrene in damaged muscle tissue and is responsible for a common type of food poisoning when the bacteria are ingested in large numbers. More recently, *C. perfringens* has been implicated in antibiotic-associated diarrhea (1, 2). This has been demonstrated in the absence of any other detectable species of diarrhea-inducing bacteria. In addition, the organism is associated with some cases of sporadic diarrhea (1, 2).

Two typing schemes are available for *C. perfringens*: serotyping (3, 9) and bacteriocin typing (4, 7, 8, 10, 11). Serotyping requires a large battery of antisera prepared against the capsular antigens of *C. perfringens*, and bacteriocin typing, as developed in our laboratory (8), requires 18 bacteriocins for strain differentiation. Recently, we (5) have described a rapid microextraction procedure for plasmids of *C. perfringens*, and it was our interest in this study to examine plasmid analysis as a means of strain differentiation and to compare the findings with those obtained by serological typing. Serological typing was done at the Central Public Health Laboratory, and plasmid typing was developed at Dalhousie University.

Fifty-two *C. perfringens* isolates were obtained from patients with diarrhea in Northwick Park Hospital, Harrow, Middlesex, England, and, occasionally, from other hospitals and environmental sources within the hospitals. Sixty-two strains were isolated during various outbreaks of *C. perfringens* food poisoning and were obtained from the Food Hygiene Laboratory, Central Public Health Laboratory, London, England. These isolates were identified as described previously (1). Serotyping of *C. perfringens* was performed as described by Stringer et al. (9), and *C. perfringens* enterotoxin from stool specimens was detected by a Vero cell assay and enzyme immunoassay as described by Borriello et al. (1). Cultures were grown at 37°C in cooked meat medium (Difco Laboratories, Detroit, Mich.) and maintained as stock cultures in this medium at room temperature.

The ability of the organisms to produce bacteriocins active against a universally susceptible strain of *C. perfringens* was screened essentially as described by Riley and Mee (6). Growth of the cultures on membrane filters (pore size, 0.22 μm; Millipore Corp., Bedford, Mass.) placed on blood agar plates permitted diffusion of bacteriocins into the agar. Removal of the membrane followed by swabbing the plates with the indicator strain permitted detection of bacteriocin activity. The plates were incubated in a Forma Scientific anaerobic glove box at 37°C.

Plasmids were extracted from *C. perfringens* as described by Mahony et al. (5). The final DNA preparation was electrophoresed in a 0.7% agarose gel for 5 h at 100 V. Ethidium bromide-stained gels were observed under UV light (Fotodyne Inc., New Berlin, Wis.) and photographed with Polaroid type 52 film or Pan film (Eastman Kodak Co., Rochester, N.Y.). Closed circular DNA plasmids used as molecular weight markers were described previously (5). Different plasmid profiles were assigned different letters.

Fifty-two *C. perfringens* isolates from hospital patients or environments were compared in this study. A diagrammatic representation of plasmid profiles and the plasmid types, numbers of isolates, and serotypes are shown in Fig. 1. Most of these isolates were obtained from Northwick Park Hospital. Forty-eight (92%) of the isolates contained plasmids; in this group, 12 serotypes and 14 plasmid profiles could be identified. Plasmid profiles D_1, D_2, and D_3 were considered to represent the predominant outbreak strain(s), and all but one of these strains were serotype 41. Each had a common 10-megadalton plasmid and two or three larger plasmids (35, 40, and 66 megadaltons for profile D_1). The largest plasmid was not detectable in plasmid profile D_2 or D_3, and the migration distances of the two large plasmids differed slightly. Five of six serotype 27 strains were plasmid type B. Most of the remaining isolates were considered one or two of

* Corresponding author.
† Present address: Campden Food Preservation Research Association, Chipping Campden, Gloucestershire GL55 6LK, United Kingdom.

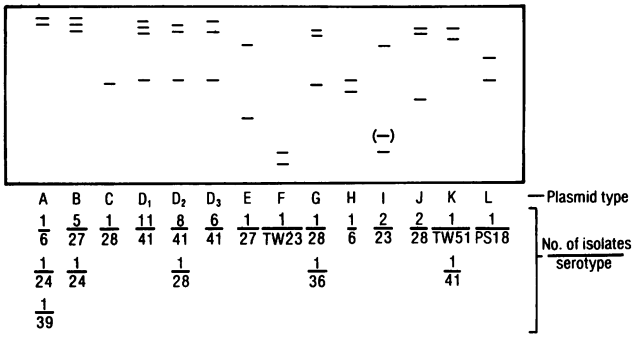

FIG. 1. Diagrammatic representation of plasmid profiles of *C. perfringens*. The plasmid profiles were obtained by electrophoresis of extracted plasmid DNA on a 0.7% agarose gel running at 100 V for 5 h. Plasmid profiles (types) were assigned letters. The number of isolates (numerator) and serotype (denominator) are listed below the plasmid type. The diagram indicates the relative mobilities of the plasmids and is not a precise representation.

a kind, as differentiated by either typing method. Six representative plasmid profiles are shown in Fig. 2.

The plasmid types, predominant serotypes, and sources of *C. perfringens* isolates obtained from patients and hospital environments are shown in Table 1. Some patients had diarrhea without detectable enterotoxin in their stools, and others had detectable enterotoxin. Isolates identified by either typing method as the predominant strains in these cases of diarrhea were found on inanimate objects in the environment of the patient as well as in the stools of the patient. Such strains were found on the hand of one patient and on the fingers of a physiotherapist. Both typing methods showed that patient 10 excreted the same strain of *C. perfringens* for 19 days and that the *C. perfringens* strain isolated from patient 1 was different from the strain isolated from the back shelf of patient 1. Plasmid type D_3 isolates obtained from various environmental sources were not found in the patients.

Numerous epidemiological observations on this outbreak have been previously reported (1), but we believe that plasmid typing provided additional information. Patient 16

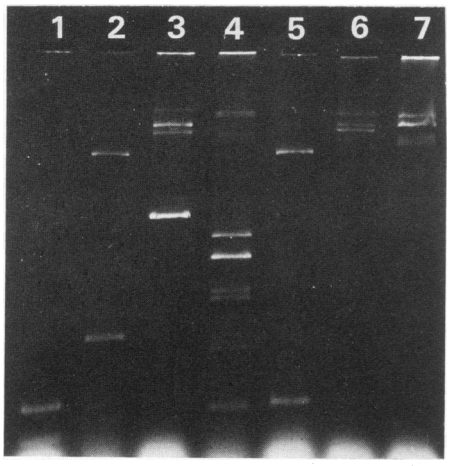

FIG. 2. Six plasmid profiles (lanes 1, 2, 3, 5, 6, and 7) shown with a control (lane 4). They represent plasmid profiles F, E, D_1, I, B, and A, respectively. The control was a composite of plasmids obtained from *Escherichia coli* V517 and RP4 and *C. perfringens* no. 28. Plasmids were electrophoresed at 100 V for 5 h.

TABLE 1. Plasmid types, predominant serotypes, and sources of *C. perfringens* isolates obtained from patients and hospital environments[a]

Plasmid type[b]	Serotype	Patient or specimen identification[c]	Ward	Source of specimen	Date of isolation
A	24	1(DT)	I	Stool	12/15/83
B	27	2(DT)	I	Stool	1/10/84
	27	2(DT)	I	Stool	1/24/84
	27	3(DT)	II	Stool	2/23/84
	27	4(DT)	II	Stool	12/21/83
	24	5(DT)	I	Stool	1/84
	27	E87	I	Patient 1, back shelf	12/21/83
C	28	6		Stool, no diarrhea	10/5/83
D_1	41	7(D)	II	Stool	11/28/83
	41	8(D)	II	Stool	11/28/83
	41	9	II	Stool	11/28/83
	41	10(DT)	II	Stool	11/9/83
	41	10(DT)	II	Stool	11/28/83
	41	11(DT)	II	Stool	9/19/83
	41	6	II	Stool	12/3/83
	41	12	I	Stool	12/16/83
	41	12(D)	I	Stool	12/20/83
	41	E7	I	Under bed, negative control area	10/25/83
	41	E43	II	Mobile commode	10/25/83
D_2	41	13(DT)	II	Stool	11/14/83
	41	13(DT)	II	Stool	11/24/83
	41	14(DT)	II	Stool	11/24/83
	41	11(DT)	II	Stool	9/21/83
	41	15(DT)	III	Stool	11/14/83
	28	16(DT)		Stool	8/22/83
	41	E1	I	Flush handle, negative control area	10/25/83
	41	E6	II	Sluice, positive control area	10/25/83
	41	E24	II	Patient 10, right hand	11/22/83
D_3	41	E30	III	Patient 15, back shelf	11/22/83
	41	E36	III	Patient 15, commode	11/22/83
	41	E68	II	Shelf, D bay	12/2/83
	41	E85	II	Bathroom	12/2/83
	41	E86	II	Bathroom	12/2/83
	41	E77	II	Fingers of physiotherapist	12/2/83
E	27	E140	IV	Floor	1/13/84
G	28	6		Stool, no diarrhea	9/30/83
J	28	17(DT)		Stool	6/6/83
	28	18(DT)		Stool	11/3/83
K	41	E55	II	Sink, B bay	12/2/83
NT	41	11(DT)	II	Stool	10/24/83
	24	19		Stool	1981

[a] All isolates were from Northwick Park Hospital except those carrying plasmids of type J, which were from Charing Cross Hospital.

[b] NT, Nontypeable.

[c] E, Environmental isolate; (D), patient with diarrhea but no detectable enterotoxin in the stool; (DT), outbreak patient with diarrhea and *C. perfringens* enterotoxin in the stool.

carried a plasmid type D_2 strain that was serotype 28 (unlike all other D_2 strains, which were serotype 41). This patient was first hospitalized in a geriatric hospital before transfer to Northwick Park Hospital. The transfer preceded the outbreak in Northwick Park Hospital, and whether the strain isolated from this patient, identified as an outbreak strain by plasmid typing but not by serology, could have initiated the subsequent outbreak poses an interesting question. Two serotype 28 isolates obtained from two patients in Charing Cross Hospital were plasmid type J and were clearly different from the outbreak strains in Northwick Park Hospital. The specificity of plasmid typing was further revealed when environmental isolate E140 (serotype 27) was identified as plasmid type E. Although serologically indistinguishable from the plasmid type B diarrhea strains from wards I and II, this strain was isolated from the floor of ward IV, an area of the hospital unrelated to the outbreak. In one instance, the absence of plasmids differentiated two serotype 24 isolates. The serotype 24 isolate obtained from patient 19 in 1981 had no detectable plasmids and was clearly different from the serotype 24 strains isolated in 1983 and 1984.

Of 62 epidemiologically unrelated food poisoning isolates, 48 (77.4%) carried plasmids (data not shown). Unlike the hospital outbreak, in which common strains were involved, there was no correlation between plasmid type and serotype among this heterogeneous collection of isolates.

A high percentage of hospital isolates produced bacteriocins or some inhibiting substance detectable with our indicator strain. Of the isolates from patients and hospital environments, 90% produced a detectable inhibitor. The only negative isolates were different from the outbreak strains, unrelated to the outbreak, or from patients without diarrhea. Thus, all strains determined to be the same strain by both plasmid and serological typing were bacteriocin producers. Only 63% of the epidemiologically unrelated strains from previous food poisoning outbreaks produced bacteriocins. This latter finding is in contrast to our observation that 2 (7%) of 27 isolates from a rural setting in India produced bacteriocins (D. E. Mahony, unpublished data). Watson et al. (11) observed that 79% of strains implicated in food poisoning outbreaks produced bacteriocins; however, only 18% of 322 isolates from the feces of healthy persons, human and animal infections, various foods, and the environment were bacteriocinogenic. The significance of these observations requires further investigation.

The data presented in this report clearly indicate that a study of plasmid profiles can be very useful in differentiating strains of *C. perfringens*. Both plasmid typing and serological typing identified the prevalent strains in a diarrhea outbreak, and in some instances more detailed information was obtained by plasmid typing. Retested strains contained the same plasmids, and a number of our laboratory strains have maintained their original plasmids for several years. Nontypeable strains (containing no plasmids) could represent a problem in plasmid typing, although the absence of plasmids may also serve as a marker among strains that have plasmids. Not all *C. perfringens* strains are serologically typeable (9), and the possibility of an outbreak caused by a nontypeable strain exists. Thus, we believe that plasmid typing offers a viable alternative to serological typing of *C. perfringens* in the epidemiological study of outbreaks of food poisoning, *C. perfringens*-associated diarrhea, or infections caused by *C. perfringens*.

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