APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1984, p. 545–549
0099-2240/84/090545-05$02.00/0

Vol. 48, No. 3

Identification of *Campylobacter coli* Isolates from Animals and Humans by Bacterial Restriction Endonuclease DNA Analysis

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Ninety-nine *Campylobacter coli* isolates were examined by bacterial restriction endonuclease DNA analysis (BRENDA) with *Hin*dIII. Isolates from poultry from the same environment had identical patterns, patterns of isolates carried by suckling piglets were generally the same as those of isolates recovered from their dams, and one human patient yielded the same BRENDA type when sampled 6 weeks later. The 14 human isolates examined produced 11 distinct BRENDA types. Forty-three *C. coli* isolates from pigs were represented by 20 BRENDA types. Ten *C. coli* isolates from the feces of gulls yielded five different BRENDA types. Thirty-two *C. coli* isolates from live chickens and processed chicken yielded five different BRENDA types. Three human isolates had identical DNA patterns; two were from brothers living in the same house, and the third was from a human with no apparent relationship to the brothers. Another human isolate was identical to a poultry isolate. None of the pig strains had DNA patterns resembling those of human strains, nor were the DNA patterns like those of any strains recovered from poultry or gulls. Four *C. coli* isolates were subcultured onto agar 23 times over a period of 45 days, and their BRENDA patterns were preserved. BRENDA shows great promise for use in epidemiological studies of *C. coli*.

Campylobacter coli was first described as *Vibrio coli* (7) and was thought to be the etiological agent of swine dysentry (6). Véron and Chatelain (36) classified the genus *Campylobacter* and renamed *V. coli* as *C. coli*. This renaming has recently been accepted by the International Committee on Systematic Bacteriology (27). The feature which distinguishes *C. coli* from *C. jejuni* is its inability to hydrolyze hippurate in the test described by Harvey (9) and Skirrow and Benjamin (30). Although *C. coli* is less frequently isolated from cases of human diarrhea than is *C. jejuni* (3, 4, 11, 14, 22, 31), there appears to be no obvious difference in disease severity for humans with either of these two species (29). Each year in New Zealand a number of acute enteritis cases in humans are found to be caused by *C. coli* (D. M. Norris, personal communication), but their source is unknown. *C. coli* is the predominant *Campylobacter* species colonizing the intestinal tract of pigs (20, 31–33). It is also sometimes found in poultry, although *C. jejuni* is much more frequently isolated from poultry (25, 31). It has been suggested by Skirrow (28) and Lior et al. (16) that pigs may constitute an important source of *C. coli* infection for humans.

Attempts have been made to develop a serotyping system for the identification of *C. jejuni* and *C. coli*. In Canada, two serotyping schemes for *Campylobacter* spp. are being developed: that of Lior et al. (17) dealing with *C. jejuni*, in which 14 to 17% of the strains examined were found to be untypable, and another system devised with both *C. jejuni* and *C. coli* in mind (21). Certain difficulties with the latter system have been encountered, and it has been suggested that plasmid typing may be necessary as an additional means of discrimination (5).

Bacterial restriction endonuclease DNA analysis (BRENDA) has already been shown to be useful for the identification of *V. cholerae* (12), and *Leptospira interrogans* serotypes (18, 24). This study was undertaken to determine the usefulness of BRENDA as a technique for studying the epidemiology of *C. coli*.

MATERIALS AND METHODS

Source of isolates. Table 1 lists the sources of 99 *C. coli* isolates examined by BRENDA. A total of 83 strains were isolated in our laboratory, 14 were isolated elsewhere from cases of human diarrhea, and 2 were type strains.

Identification. Campylobacters were identified on the basis of colony appearance, microscopic morphology and motility, positive catalase reaction (36), and oxidase activity determined by the method of Kovacs (15). The ability or inability to hydrolyze hippurate, detected with ninhydrin, was tested by the method described by Skirrow and Benjamin (30). Sensitivity to nalidixic acid and cephalothin (30 μg) was determined by a disk diffusion test. The absence of a clear zone of inhibition was reported as resistance (13). Growth at 25 and 42°C was also recorded. Table 2 summarizes the features by which catalase-positive *Campylobacter* spp. were distinguished.

Preparation of DNA. *C. coli* was harvested in 10 ml of phosphate-buffered saline (10 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1 mM KH_2PO_4 [pH 7.2 to 7.4]) from blood agar plates which contained 7% defibrinated sheep blood and 0.05% FBP supplement (10) and which had been incubated at 42°C under microaerophilic conditions for 24 h. One plate yielded sufficient organisms (10^9 to 10^{10}) for the DNA analysis of each strain. Each culture was centrifuged at 10,000 × *g* for 30 min (Sorvall SS-34 centrifuge) and resuspended and washed in phosphate-buffered saline. The remaining methods we used have been described previously (18), apart from the following modifications: sodium perchlorate was added as 350 μl of a 5 M stock solution, and fluorimetry was performed on all samples with calf thymus DNA (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 0 to 1 μg/ml for construction of a standard curve.

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TABLE 1. Sources of 99 *C. coli* isolates tested by BRENDA

Source	Sample	No. of isolates
Humans	Rectal swabs and feces	14
Pigs	Rectal swabs	41
Poultry	Cloacal swabs	20
Processed chicken	Whole washing rinse	12
Southern black-backed gulls (*Laurus dominicanus*)	Fresh feces	10
NCTC 11366^T	Pig feces	1
NCTC 11353	Pig placenta	1

Restriction endonuclease digestion of DNA. Bacterial DNA (2.0 μg) was digested to completion for 1 h at 37°C with restriction endonuclease *Hin*dIII. Initial studies with *Eco*RI showed that this enzyme did not digest campylobacter DNA (unpublished data). *Hin*dIII was purified in this laboratory from *Haemophilus suis* (ATCC 19417) by the method of Greene et al. (8). The digestion mix contained 60 mM NaCl, 10 mM $MgCl_2$, 10 mM Tris-hydrochloride, and 100 μg of bovine serum albumin (pH 7.5) per ml in a final volume of 100 μl. Bacteriophage *c*I857 S7 made from a lysogenic strain of *Escherichia coli* (19) with the DNA extracted by the method of Younghusband and Bellett (38) was used as a reference marker for each series of digests.

Gel electrophoresis and photography. The methods used were those described by Marshall et al. (18). Electrophoresis was maintained for 3 to 4 h at 5 V/cm in 0.7% agarose gels until the bromophenol blue used as a tracking dye had traveled anodically 12.5 cm.

RESULTS

A total of 235 *Campylobacter* isolates from humans with diarrhea were received from two major medical laboratories; of these, 14 (6%) were *C. coli*. One laboratory supplied 47 *Campylobacter* isolates, and 6 (13%) of these were *C. coli*; the other laboratory supplied 188 *Campylobacter* isolates, and 8 (4%) of these were *C. coli*. The 14 isolates produced 11 different BRENDA patterns. Of the 14 human isolates, 3 had the same pattern; 2 of these were from brothers (Fig. 1, lanes 1 and 2) living in the same house, and the third was from a human apparently unrelated to the brothers. Another two unrelated human isolates yielded identical BRENDA patterns (not illustrated). A further two human isolates were found to produce identical patterns, but these were subsequently found to be from the same person who had, for medical reasons, been resampled after 6 weeks. Only one of these two isolates was included in the data reported above, but both are shown in Fig. 1, lanes 3 and 4. This BRENDA pattern was also found in 17 isolates from 20 chickens from one poultry flock (Fig. 1, lane 5). Another BRENDA pattern was found in three isolates obtained from two different flocks owned by the same person (Fig. 1, lanes 6 and 7). Twelve isolates were obtained from three batches of processed chicken. Examples of the patterns produced by two of these isolates are shown in Fig. 2, lanes 6 and 7. Each batch was contaminated with *C. coli* of the same BRENDA pattern, but the strains from each batch were different.

A total of 18 different BRENDA types of *C. coli* were demonstrated by the 41 isolates from pigs, and another two BRENDA types were demonstrated by the two type cultures NCTC 11353 and NCTC 11366. The pattern of NCTC 11366 is shown in Fig. 2, lane 5. The BRENDA types of *C. coli* carried by newborn suckling piglets were in most cases identical to those of *C. coli* carried by the sow which these piglets suckled (Fig. 1, lanes 8 and 9). In a few cases, the piglets carried a BRENDA type identical to that carried by another sow, usually one to which the suckling piglets had access. The two type cultures yielded different patterns, unlike those of any other isolates as well. Two other examples of pig isolates are shown in Fig. 2, lanes 3 and 4.

The 10 *C. coli* isolates from gulls produced five BRENDA types different from any BRENDA types previously recovered. Two of the patterns from gulls are shown in Fig. 2, lanes 8 and 9.

Four *C. coli* isolates representing different BRENDA types were subcultured onto agar 23 times over a period of 45 days. The BRENDA pattern of each isolate was still preserved at the conclusion of this experiment (Fig. 3).

DISCUSSION

C. coli is mainly associated with pigs. Ninety percent of pigs over 6 weeks of age carry *Campylobacter* spp., and almost all of these are *C. coli* (C. K. Kakoyiannis, D. K. Blackmore, and R. B. Marshall, Abstr. Proc. N.Z. Microbiol. Soc. 1983, 17.6, p. 81). On the other hand, only 14% of chickens carry *C. coli* (C. K. Kakoyiannis, D. K. Blackmore, and R. B. Marshall, Abstr. Proc. N.Z. Microbiol. Soc. 1983, 17.6, p. 81). In this study, isolates from live poultry produced two BRENDA types, and another three types were found in processed chicken. Pig isolates from a pig-rearing unit produced 18 different BRENDA types. The BRENDA patterns of the two type cultures, which were of porcine origin, were different from each other and unlike those of any of the other isolates. In spite of this large representation of isolates (43) from pigs, none of the 20 BRENDA patterns resembled any of the BRENDA patterns from the 14 human isolates. This result supports recent findings that *C. coli* serotypes from pigs are uncommon among humans (20). The finding that 17 of the poultry isolates had a BRENDA pattern identical to that of human isolate is interesting, but because these isolates came from different areas a direct epidemiological link between them cannot be inferred. Persistent infection (37) is the most likely reason for the recovery in a human of *C. coli* of the same BRENDA type on two different occasions ca. 6 weeks apart. This finding, together with the observed stability of BRENDA patterns after repeated laboratory subculturing, gives us confidence that *C. coli*, like *C. jejuni* (unpublished data), maintains a stable BRENDA pattern for a period of months and probably for much longer. Confidence in the stability of BRENDA patterns is further substantiated by the finding in poultry that *C. coli* isolated from the same environment, whether from live flocks or processed chicken, always yielded identical BRENDA patterns. That suckling piglets

TABLE 2. Differentiation of catalase-positive intestinal campylobacters (13, 30)

Organism	Growth[a] at:		Sensitivity[b] to 30 μg of:		Hippurate hydrolysis[c]
	42°C	25°C	Naladixic acid	Cephalothin	
C. fetus subsp. *fetus*	−, (+)	+	R	S	−
C. jejuni	+	−	S	R	+
C. coli	+	−	S	R	−
NARTC[d]	+	−	R	R	−

[a] +, Positive; −, negative; (+), few strains grow at 42°C (30).
[b] R, Resistant; S, sensitive.
[c] +, Positive; −, negative.
[d] NARTC, Nalidixic acid-resistant thermophilic campylobacter.

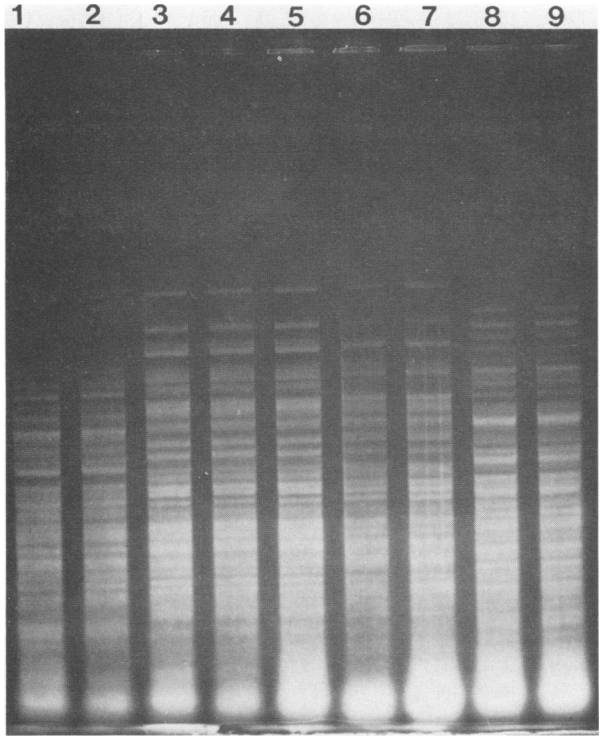

FIG. 1. BRENDA patterns produced by *Hin*dIII digestion of *C. coli* isolates from humans, chickens, and pigs. Lanes: 1 and 2, isolates from brothers living in the same house and suffering from diarrhea; 3 and 4, isolates taken 6 weeks apart from a patient with diarrhea; 5, a chicken isolate with a pattern identical to that of the human isolates shown in lanes 3 and 4; 6 and 7, isolates from chickens on the same farm; and 8 and 9, isolates from a sow and her piglet, respectively.

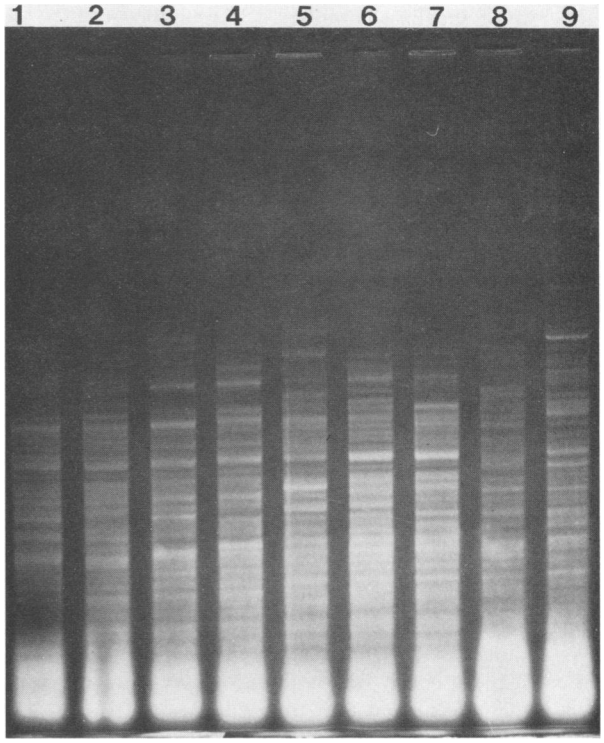

FIG. 2. BRENDA patterns produced by *Hin*dIII digestion of *C. coli* isolates from humans, pigs, processed chicken, and gulls and of NCTC 11366. Lanes: 1 and 2, human isolates; 3 and 4, pig isolates; 5, NCTC 11366; 6 and 7, processed chicken isolates; and 8 and 9, gull isolates.

usually become colonized by *C. coli* of the same BRENDA type as that carried by their dam is expected. In those cases in which a piglet was carrying a *C. coli* isolate with a BRENDA type different from that carried by its dam, the type was similar to the type carried by a nearby sow to which the piglet had access. Colonization of the piglet with *C. coli* of this different type could be due to the carriage of both types by its dam, to the piglet suckling the other sow (unpublished data), or to general environmental contamination (26). These results indicate that BRENDA typing will be of value in epidemiological studies aimed at determining likely sources of human or animal infection. BRENDA patterns from leptospires remain stable for many years, and reference strains which have been held in laboratories in different countries for decades still generate identical BRENDA patterns (24).

The finding that 41 isolates from one pig-rearing unit were represented by 18 different BRENDA types contrasts with the finding in poultry, for which only 1 BRENDA type was found in all birds sampled in any one flock. The representation of serological types in these two species shows a similar distribution (20). The different management regimes and the frequent introduction of new animals to the pig-rearing unit may in part explain this phenomenon. The conflicting results of experimentally infecting pigs with *C. coli* reported by different countries (1, 2, 6, 23, 34, 35) could possibly be explained by the fact that different strains, which had various degrees of pathogenicity, were used. BRENDA typing could be used to more precisely identify such strains.

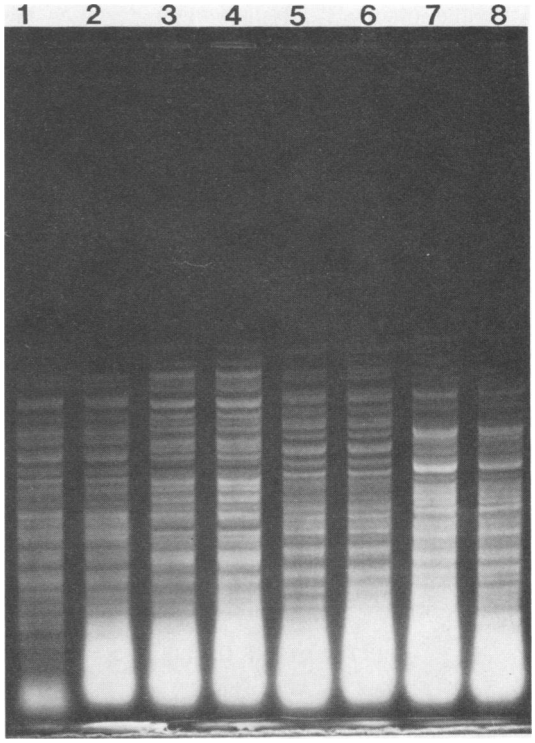

FIG. 3. BRENDA patterns produced by digestion of *C. coli* isolates with *Hin*dIII before and after subculturing. Lanes 2, 4, 6, and 8 show the patterns produced after 23 subcultures. The respective original isolates are shown in lanes 1, 3, 5, and 7.

In view of the large number (41) of different BRENDA types of *C. coli* already identified, it is unlikely that a serological method of classification would be as sensitive, and therefore as useful, in epidemiological investigations. Karmali et al. (14) stated that serology does not necessarily imply identity among strains belonging to the same serotype and suggest that additional differential markers may be required. BRENDA typing could be used either on its own or in conjunction with serotyping as a means of strain identification. The BRENDA method could possibly be made even more sensitive by applying different restriction enzymes to those organisms which show identical patterns after digestion with the initial enzyme; however, to date we have not found, when using *Hin*dIII, any identical strains which yielded a different pattern with a second enzyme. Organisms which produce pronounced differences in DNA patterns clearly have a different arrangement of base pairs within their chromosomes and cannot be considered as having been derived from the same parent stock.

Minor differences between DNA electrophoretic patterns of different *C. coli* strains may be due not to chromosomal DNA but to the presence or absence of plasmids. Because the plasmid components of different strains of *C. coli* and *C. jejuni* appear to be relatively stable (5), these minor differences should be taken into account during epidemiological studies. If there is any doubt about the presence or absence of plasmids, a gel can be run with the extracted DNA without subjecting it to restriction enzyme digestion. Although not a sensitive method for the detection of plasmids of large molecular weight, it does enable these plasmids with no cutting sites which contribute to the BRENDA pattern to be detected.

BRENDA may not be sufficiently simple to become established in all routine diagnostic laboratories. When a detailed epidemiological study is being undertaken, however, this technique can easily be established and will, we believe, add to an understanding of the natural history of the enteric *Campylobacter* spp. by providing precise identification of the different isolates. The function of a test such as this is rather different from that of serological typing, where precision and sensitivity may be sacrificed for ease of operation. If the technique can be simplified and produced in a kit form and if the results can be stored in a computer, BRENDA could become part of a laboratory's routine identification procedures for *Campylobacter* spp. and also for many other types of bacteria. Development of the technique along these lines is currently being carried out in our laboratory. Although these studies are only of a preliminary nature, it is believed that they demonstrate the potential value of BRENDA typing of *Campylobacter* spp. in epidemiological investigations.

ACKNOWLEDGMENTS

This work was supported by grant 82/89 from the New Zealand Medical Research Council.

We also thank the National Health Institute for assistance and the Hamilton Medical Laboratory for providing the human isolates.

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