Restriction Endonuclease DNA Analysis of Clostridium difficile

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HindIII restriction enzyme digests of genomic DNA from nine distinct strains of Clostridium difficile were undertaken, and the results were related to those of a previously established typing method based on [35S]methionine-labeled protein profiles. Each of the typed strains identified by its protein profile could also be distinguished by its unique DNA digestion pattern. Analysis of strains isolated from 10 patients during a hospital outbreak of antibiotic-associated colitis revealed identical DNA profiles, confirming a single strain as the source of cross-infection. Characterization of isolates from worldwide sources revealed similar digestion patterns within the same strain type. Restriction endonuclease DNA analysis provides a sensitive and useful technique for studying the epidemiology of C. difficile.

The association of Clostridium difficile with pseudomembranous colitis and antibiotic-associated colitis is well established (1, 4, 7). Several reports of clusters of cases of C. difficile-related outbreaks in hospitals (5, 8, 12) have necessitated the development of new typing methods to monitor cross-infection and nosocomial acquisition of the organism (3, 13-15). We previously reported a typing scheme for C. difficile that was based on the incorporation of [35S]methionine into cellular proteins, their separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and visualization by autoradiography (14, 15). All strains tested incorporated the [35S] radiolabel. The distinctive radiolabeled protein profiles obtained were designated into nine types: A to E and W to Z. This typing scheme has been successfully applied in epidemiological studies of C. difficile-associated diarrheal disease in our hospital (5, 14). In this study, we used restriction endonuclease DNA analysis of C. difficile to develop an alternative method for studying the epidemiology of C. difficile and to confirm the distinctions between the various strain types.

Representative strains of types A to E and W to Z (14, 15) were used. Ten strains previously identified as type X were obtained from different patients during a documented outbreak of antibiotic-associated colitis at St. Bartholomew's Hospital (5). Other type D and E strains were obtained from S. Arnon, Berkeley, Calif.; C. E. Nord, Stockholm, Sweden; and D. Burdon, Birmingham General Hospital, Birmingham, United Kingdom.

Strains were cultured and identified as previously described (14). For the preparation of genomic DNA, a sweep of C. difficile from a blood agar plate was transferred to 50 ml of prereduced Nakamura broth (10) and incubated at 37°C overnight under anaerobic conditions. The bacteria were harvested by centrifugation and washed in TE (10 mM Tris hydrochloride, 1 mM EDTA [pH 8.0]) buffer. The pellet was suspended in 3 ml of 1 M sucrose and 3 ml of 10 mg of lysozyme (egg white grade III; Sigma Chemical Co., Poole, United Kingdom) per ml in TE buffer and incubated for 1 h at 37°C. The solution was centrifuged in an MSE 8 x 50 ml rotor at 10,000 × g for 10 min at 4°C, and the pellet was suspended in 1.5 ml of 50 mM Tris hydrochloride-0.5 mM EDTA (0.25 M) (pH 8.0) and 0.125 ml of a pronase (Sigma Chemical Co.) solution (20 mg/ml of water) and incubated for 30 min at 37°C. The cells were lysed by the addition of 0.5 ml of a 10% solution of N-lauroylsarcosine (Sigma Chemical Co.) and incubated at 37°C for 20 min to ensure complete lysis. Sterile water (2.5 ml) was added to each cleared lysate, which was then incubated for 30 min at 37°C with 50 μl of RNase (10 mg/ml). The lysates were then treated successively with 5 ml each of phenol, phenol-chloroform, and chloroform. Finally, the DNA was precipitated with ethanol, pelleted, dried under vacuum, and dissolved in 500 μl of TE buffer at a concentration of 0.7 mg/ml. DNA (3 μg) was digested to completion by incubation at 37°C with 15 U of the appropriate restriction endonuclease for 1 h under the conditions recommended by the manufacturer (Northumbria Biochemicals Limited, Cramlington, United Kingdom). The enzymic reactions were terminated by heating the samples at 68°C for 10 min. The DNA fragments were separated by electrophoresis in a horizontal gel containing 0.5% agarose (nucleic acid grade; Pharmacia Ltd., Milton Keynes, United Kingdom) in TB (89 mM Tris hydrochloride, 89 mM boric acid, 2 mM EDTA [pH 8.0]) electrophoresis buffer, containing 1 μg of ethidium bromide per ml. To reduce the complexity of the DNA patterns, the agarose gel was electrophoresed under conditions which preferably separated out larger DNA fragments in the range 7 to 25 kilobases. The gel was run overnight at 60 V and photographed through a Kodak (Wratten no. 23) filter with a Polaroid MP-4 land camera. Banding patterns in all digests were reproducible.

The restriction endonucleases BamHI, EcoRI, SalI, and SmaI gave insufficient banding to distinguish between strains. However, HindIII produced highly resolved DNA restriction fragments and was selected as the most suitable enzyme. HindIII digestions of DNA from C. difficile strains representative of the nine types, A to E and W to Z, are shown in Fig. 1. Each of the nine distinct strains can be differentiated on the basis of its characteristic DNA pattern on agarose gel electrophoresis.

The 10 isolates from different patients in an outbreak of antibiotic-associated colitis were also analyzed. The HindIII restriction patterns for all 10 human isolates were indistinguishable (Fig. 2), as was the case using the restriction endonuclease XbaI (data not shown). These isolates had all

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been typed as X strains, had identical antibiograms, and were plasmid free (unpublished data), strongly supporting the view that this common isolate was the epidemic strain.

Further studies on C. difficile strains from four geographical areas showed similar patterns for four type D strains (Fig. 3, lanes 5 to 8) and three type E strains (lanes 9, 10, and 12) and a clearly distinguishable pattern for the fourth untyped strain (lane 11). Also shown in Fig. 3 are the distinct HindIII digests of DNA from Clostridium tetani, Clostridium histolyticum, Clostridium sordellii, and Clostridium novyi (lanes 1 to 4).

Restriction endonuclease DNA analysis with HindIII appears to be a very sensitive method for differentiating C. difficile isolates and should be a useful tool for identifying sources and tracing routes of transmission of C. difficile-associated infections. Recently, Kuijper et al. reported the application of this method in a small outbreak of C. difficile-induced disease (6).

Although only a limited number of strains were studied, isolates from widely differing geographical sources appear to have similar DNA profiles. Similar findings have been reported for strains of Campylobacter jejuni (2) and Corynebacterium diphtheriae (11). However, the use of a single restriction enzyme, such as HindIII, may not enable us to distinguish subtle differences in DNA sequence.

Other reported typing schemes for C. difficile have relied on serotyping (3) and bacteriophage typing (13) as methods for distinguishing strains. Plasmid analysis of C. difficile has proved unsuccessful, since the organism has been reported to generally lack plasmid DNA (9). All these techniques have their limitations in sensitivity and specificity: restriction endonuclease DNA analysis appears to be a more direct and definitive approach to differentiating organisms and should provide a useful epidemiological marker for the study of C. difficile-associated diarrheal disease.

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LITERATURE CITED


