Typing of *Clostridium difficile* by Western Immunoblotting with 10 Different Antisera

HARU KATO,†† JOSEPH J. CAVALLARO,¹ NAOKI KATO,† SUZETTE L. BARTLEY,³ GEORGE E. KILLGORE,³ KUNITOMO WATANABE,² AND KAZUE UENO²

Nosocomial Pathogens Laboratory Branch, Hospital Infections Program, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333, and Institute of Anaerobic Bacteriology, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu, 500 Japan²

Received 4 August 1992/Accepted 29 October 1992

Western blotting (immunoblotting) with antisera against each of 10 reference serogroups was evaluated as a means of typing *Clostridium difficile*. A total of 164 clinical isolates of *C. difficile* were tested. Variations in band profiles in each serogroup were used to type isolates into subserogroups. This technique was useful for an epidemiological investigation.

Outbreaks of *Clostridium difficile*-associated diarrhea in hospitalized patients are well documented (3, 5, 7, 8, 10). The availability of a simple, rapid, reproducible, and highly discriminatory typing scheme for *C. difficile* would greatly aid studies of both the epidemiology and pathogenicity of *C. difficile*. Various typing methods have been reported to date (3, 5, 7–10, 13). Of these, Western blotting (immunoblotting) results have been reported to be relatively simple to interpret and the test is sufficiently sensitive for typing of epidemiologically related *C. difficile* isolates (9, 10). The studies used Western blotting as the typing method and antiserum prepared against one strain of *C. difficile* (10) or antiserum prepared against two clinical isolates (9). However, typing into serogroups that are defined by a slide agglutination test (5, 6) is likely more beneficial in terms of the epidemiology and pathogenicity of *C. difficile*. Therefore, in the present study, we prepared antiserum against the 10 reference serogroups of *C. difficile*. These antisera were used to type clinical isolates by the Western blotting technique.

Ten reference strains of *C. difficile* representing the following 10 different serogroups (5, 6) were obtained from the American Type Culture Collection: A, ATCC 43594; B, ATCC 43593; C, ATCC 43590; D, ATCC 43597; F, ATCC 43598; G, ATCC 43599; H, ATCC 43600; I, ATCC 43601; K, ATCC 43602; X, ATCC 43603. A total of 164 *C. difficile* strains isolated from clinical specimens were tested. Of the 164 strains, 114 were isolated from epidemiologically unrelated patients; the strains were received by the Anaerobic Bacteria Branch, Centers for Disease Control, between 1984 and 1986 and were from various sources. The remaining 50 strains were isolated from 36 patients associated with a presumed outbreak of *C. difficile*-associated colitis or diarrhea at a hospital in New York in 1989 and 1990. All 50 isolates were recovered from fecal specimens from symptomatic adult patients. *C. difficile* was isolated and identified by standard procedures (1, 2). Antiserum were prepared by immunizing New Zealand rabbits by the method described previously (5) by using each of the reference strains of the 10 different serogroups. Protein antigen was prepared by EDTA extraction of *C. difficile* (13). Electrophoresis of EDTA

* Corresponding author.
† Present address: Institute of Anaerobic Bacteriology, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu, 500 Japan.
were nontypeable. When we used homologous antiserum, there were variations in immunoblot patterns among the strains belonging to the same serogroup, with bands located beyond the region of 60 kDa. These variations showed reproducibility after several subcultures. Therefore, these differences were used to further type the isolates into subserogroups within a serogroup. Representative immunoblot patterns of serogroup K isolates with homologous serogroup K antiserum are shown in Fig. 2; depending on the locations of the bands of greater than 60 kDa, the isolates tested displayed eight different profiles. Of the 114 unrelated clinical isolates, 68 were further typed into 22 subserogroups.

Fifty C. difficile isolates were obtained from 36 patients involved in what was believed to be a hospital outbreak. A total of 34 of these isolates were typed as serogroup G and of these, 33 (66%) were typed as subserogroup G-1; these 33 strains were isolated from 28 (78%) of the 36 patients. C. difficile was repeatedly isolated from more than two different fecal specimens from nine patients. In three of these nine patients, the serogroups of the isolates changed from one to another during their hospitalization.

In the present study, the application of immunoblotting with rabbit antisera prepared against each of the 10 reference serogroup strains consistently demonstrated the presence of easily identifiable serogroup-specific protein bands in the region of 30 to 60 kDa. The method was able to differentiate between the 10 serogroups of C. difficile and to do so reproducibly. Common bands among C. difficile strains were not demonstrated (4). In our study, the most commonly recovered strains from epidemiologically unrelated isolates belonged to serogroups A, G, H, and K. These results confirm the previous observations of Toma et al. (11). In addition, the present study showed additional minor bands in the region beyond 60 kDa. These bands were reproducible and useful in typing the different isolates into subserogroups.

The results of our laboratory investigations supported the fact that there was an outbreak of C. difficile infection among the hospitalized patients we examined; one of the serogroup G outbreaks, subserogroup G-1, was recovered from 78% of patients; on the other hand, strains of this subserogroup were isolated from only 7.0% of the patients not involved in the outbreak. We concluded that this subserogroup strain was the causative agent of the outbreak.

The 10 serogroups of C. difficile were characterized (5, 6), and the reference strains are available from the American Type Culture Collection. Once antisera against the reference serogroup strains have been produced which cannot easily be performed in clinical laboratories, Western blotting seemed to be a potent method of typing C. difficile and could offer a means of exchanging typing results among laboratories. One limitation of the Western blotting technique for typing is the number of antisera needed for serogrouping. In the present study, some isolates could not be typed with the available antisera. Establishment of additional serogroups of C. difficile is required to make new antisera and to make the Western blotting technique more useful for typing.

In conclusion, we demonstrated that the Western blotting technique is a sensitive and viable method for typing C. difficile, although we found appreciable numbers of strains that could not be typed. The method was able to differentiate different C. difficile isolates and allow the determination of the causative agent of an outbreak. The relationship and correlation of serogroups or subserogroups to toxigenicity, pathogenicity, and clinical symptomology of C. difficile infections warrant additional studies.

REFERENCES


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