JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1981, p. 349
0095-1137/81/090349-01$02.00/0

Vol. 14, No. 3

Detection of *Clostridium difficile* Toxin by Counterimmunoelectrophoresis: a Note of Caution

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Received 18 March 1981/Accepted 27 April 1981

Recent methods for detection of *Clostridium difficile* toxin by counterimmunoelectrophoresis might lead to errors. False-positives may be attributable to soluble cell surface antigens reacting with impure antitoxin.

Several workers have recently attempted to develop a counterimmunoelectrophoresis (CIE) method for the detection and quantitation of *Clostridium difficile* cytotoxin in both fecal and culture filtrates (3, 4). If valid and successful, such a system would have obvious advantages over the cytotoxic assays that are currently in use for investigation of antibiotic-associated colitis. We are convinced, however, that the antigen that is being detected by CIE and reported in these two papers is not necessarily the *C. difficile* toxin and could be a cell surface antigen of *C. difficile*. Our evidence for this is as follows.

(i) We have recently shown that *C. difficile*, *Clostridium sordellii*, and *Clostridium bifermentans*, but no other clostridial species tested, have at least one surface carbohydrate antigen in common (1). Welch et al. (4) show that cytotoxic assay-negative strains of *C. sordellii* and *C. bifermentans* are both positive by CIE. Similarly, in both papers (3, 4) all *C. difficile* strains tested are CIE positive, although some are cytotoxic assay negative.

(ii) Although we are unable to test any of the conditions available in the United States, our own *C. difficile* antitoxin and the *C. sordellii* antitoxin supplied by the Wellcome Research Laboratories (Beckenham, England) have antibodies to the common cell surface antigens. Commercial preparations of antitoxin are usually raised against relatively impure toxin preparations.

(iii) In attempting to purify the *C. difficile* toxin by the method of Rolfe and Finegold (2) we found that the cell surface carbohydrate antigen copurified with the toxin.

(iv) *C. difficile* in culture, especially if any cell lysis has occurred, releases much cell surface antigen into the medium.

While agreeing with the other authors (3, 4) as to the importance of identification of toxigenic strains of *C. difficile* for the management of cases of antibiotic-associated colitis, we suggest the need for caution in interpreting the results of CIE studies in this area. The absorption of the antitoxin with whole cells of *C. sordellii*, *C. bifermentans*, or *C. difficile* will remove the anti-surface immunoglobulins and may allow more specific results. Similarly, antitoxin raised against absolutely pure toxin will give specific results.

There is still much that is not understood about the roles of both toxigenic and nontoxigenic strains of *C. difficile* as enteropathogens; possibly erroneous reports of toxin-positive strains will increase our confusion.

Our studies on *C. difficile* are supported by the Scottish Home and Health Department, grant no. K/MRS/50/C247.

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