JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1985, p. 638–640
0095-1137/85/040638-03$02.00/0

Vol. 21, No. 4

Detection of *Haemophilus influenzae* Type b Antigenuria by Bactigen and Phadebact Kits

LOURDES RIERA

Microbiology Laboratory, Department of Pathology, Michael Reese Hospital, Chicago, Illinois 60616

Received 5 September 1984/Accepted 19 December 1984

Two commercially available reagents, latex particles (Bactigen) and *Staphylococcus aureus* suspensions (Phadebact), were compared for the detection of the capsular polyribitol phosphate antigen of *Haemophilus influenzae* type b in 18 pediatric patients with proven infections due to *H. influenzae* type b. Whereas both tests nearly equally detected the antigen in the first urine specimens from the patients, the latex test remained positive significantly longer than did the Phadebact test for serial urine specimens. We conclude that the Bactigen test is slightly more sensitive than the Phadebact test for detecting urinary *H. influenzae* type b antigen.

The agglutination of antibody-coated particles by bacterial antigens in body fluids has been shown to be an effective technique for the early diagnosis of some infections caused by *Haemophilus influenzae* type b (2, 3, 5, 6, 10; J. I. Ward, H. W. Clegg, R. Wasserman, G. Rosenberg, and G. R. Siber, Pediatr. Res. **15**:624, 1981, [abstr. no. 1088]). The commercially available reagents for these tests are suspensions of either *Staphylococcus aureus* bearing antiboby combined with staphylococcal protein A or latex particles bearing adsorbed antibody. In infections caused by *H. influenzae* type b, these reagents have detected the specific polyribitol phosphate antigen of this species in the cerebrospinal fluid, blood serum, and urine of patients. This paper reports an investigation of the comparative diagnostic utility of these reagents for the detection of urinary *H. influenzae* type b antigen.

Methods. We studied 18 patients, aged 1 month to 10 years, with *H. influenzae* type b infections. Twelve of these patients had meningitis, documented by positive blood or cerebrospinal fluid cultures. In addition, two patients with pneumonia, two with epiglottitis, and two with cellulitis had positive blood cultures. At intervals of 1 to 3 days, we obtained serial urine specimens collected at random hours. For controls, we obtained 27 urine and 10 cerebrospinal fluid specimens from 27 pediatric patients with infectious diseases of various etiologies, including 12 patients with meningitis due to *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Mycobacterium tuberculosis*, or *Escherichia coli* and 1 patient with aseptic meningitis.

Each specimen of cerebrospinal fluid or urine was tested for *H. influenzae* type b antigen by its ability to cause agglutination of latex spheres bearing adsorbed anti-*H. influenzae* type b antibody (Bactigen, *H. influenzae* type b; Wampole Laboratories, Div. Carter-Wallace, Inc., Cranbury, N.J.) and of *S. aureus* suspensions bearing this antibody combined with staphylococcal protein A (Phadebact; Pharmacia Diagnostics, Piscataway, N.J.). Tests were performed according to the manufacturers' instructions. Specimens were tested shortly after receipt in the laboratory or were stored at −20°C. We routinely heated the urine specimens at 100°C for 3 min before performing the tests. Urine specimens that gave negative results were tested again after concentration by pressure filtration through a Minicon B15 filter (Amicon Corp., Lexington, Mass.). Specimens that gave a positive reaction were serially diluted in twofold steps with phosphate-buffered saline solution (pH 7.2, 0.1 M PO_4, 0.15 M NaCl) and tested by the latex procedure to determine the maximum dilution that gave a positive test. Collection of urine was continued at approximately 2- to 4-day intervals until the tests with concentrated urine specimens were negative or the patient was discharged.

Results. For 14 of 15 patients with confirmed *H. influenzae* type b infections, tests with either reagent were positive on unconcentrated urine specimens obtained within 2 days of admission, except for one patient whose coagglutination test was positive only after concentration of the specimen. One patient with epiglottitis had negative tests with both reagents. In two other patients with epiglottitis and pneumonia, respectively, whose first urine specimens were obtained 3 days after admission, the latex tests were positive but the coagglutination tests were negative even with concentrated specimens. In another patient with meningitis, the coagglutination test on the first urine specimen, obtained 5 days after admission, was positive only after concentration, whereas the latex test was positive without this additional step.

All tests with the 27 control specimens were negative, even after the specimens were concentrated. The mean duration of type b antigenuria in serial specimens from 15 patients who were followed until both tests became negative was greater with the latex test than with the coagglutination test (Table 1). The statistically significant difference in duration of detectable antigenuria in patients with either meningeal or nonmeningeal infections confirmed the ability of the latex test to detect lower concentrations of the *H. influenzae* type b antigen.

We determined the titer of the latex test in serial urine specimens from 12 patients with *H. influenzae* type b meningitis and 6 patients with nonmeningeal infections. Figure 1 shows the generally higher initial titers and the longer duration of positive urinary latex tests for the patients with meningitis. The geometric mean latex titer of specimens obtained during the first 2 days of hospitalization from 10 patients with meningitis was 1:549. After the first 3 to 7 days, the titers declined progressively. The few patients with nonmeningeal infections had generally low titers of positive latex tests.

Discussion. Initial studies of coagglutination and latex tests demonstrated their ability to detect *H. influenzae* type b antigen in body fluids, including urine (5, 9). These studies

TABLE 1. Duration of *H. influenzae* type b antigenuria in patients with *H. influenzae* type b infections

Test	Days (± SEM) until negative test[a]	
	Meningitis[b]	Nonmeningeal infection[c]
Latex agglutination	19.9 ± 2.3	8.6 ± 1.9
S. aureus coagglutination	13.5 ± 1.4	3.8 ± 1.8

[a] Days until a negative test was obtained with a concentrated urine specimen.

[b] $n = 10$. Excludes one patient (a, Fig. 1) who had clinical evidence of relapse in the hospital. $P < 0.001$ (Sign test).

[c] $n = 5$. Includes two patients, with epiglottitis and pneumonia, who had negative *S. aureus* tests on their initial specimens, obtained three days after entry. $P < 0.031$ (Sign test).

were made with nonstandardized reagents prepared in the laboratories of the investigators. With the advent of commercial latex and coagglutination reagents, comparative tests on the cerebrospinal fluids of patients showed them to be similarly efficacious and specific in detecting *H. influenzae* type b meningitis (2, 3, 6, 8, 10). However, in a recent study, the latex test detected as little as 0.2 ng of *H. influenzae* type b capsular antigen per ml, whereas the limit of detectability by the coagglutination test was 25 ng/ml (6). Correspondingly, for patients with meningitis, the latex test was positive for 22 specimens of cerebrospinal fluid, whereas the coagglutination test was positive for only 19. For patients with nonmeningeal type b infections, latex tests for urinary antigen were positive for 15 patients, but coagglutination tests were positive for only 4. The foregoing evidence of the superior efficacy of the latex test is supported by our finding that it detected urinary excretion of *H. influenzae* type b antigen for a longer time than did the coagglutination test.

The ability of the current commercially available latex test to detect lower concentrations of urinary type b antigen should make it the preferred reagent for the diagnosis of *H. influenzae* type b infections, especially for nonmeningeal infections and for patients who previously have been treated with antibiotics. Our finding of positive latex and negative coagglutination tests on urinary specimens from three patients with nonmeningeal infections supports this concept. For patients with presumptive *H. influenzae* type b pneumonia but without confirmatory blood cultures, latex tests on urinary specimens have afforded evidence of etiology (Ward et al., Pediatr. Res. **15**:624, 1981 (abstr. no. 1088]). Similarly, for two patients, aged 11 months and 3.5 years, with pneumonia (not cited in our results), we found positive urinary latex tests for *H. influenzae* type b antigen.

Antigens from some strains of *E. coli* and *S. pneumoniae* may cross-react with antisera to *H. influenzae* type b (1, 7, 8). In clinical practice this is an infrequent problem (4).

The evidence indicates that tests for urinary *H. influenzae* type b antigen with either of the two commercially available reagents that we examined may be useful in the diagnosis of *H. influenzae* type b infections. However, the latex test appears to be the more useful of the two in view of its higher incidence of positive results in acute *H. influenzae* type b infections and the longer duration of positive results in tests of serial urine specimens.

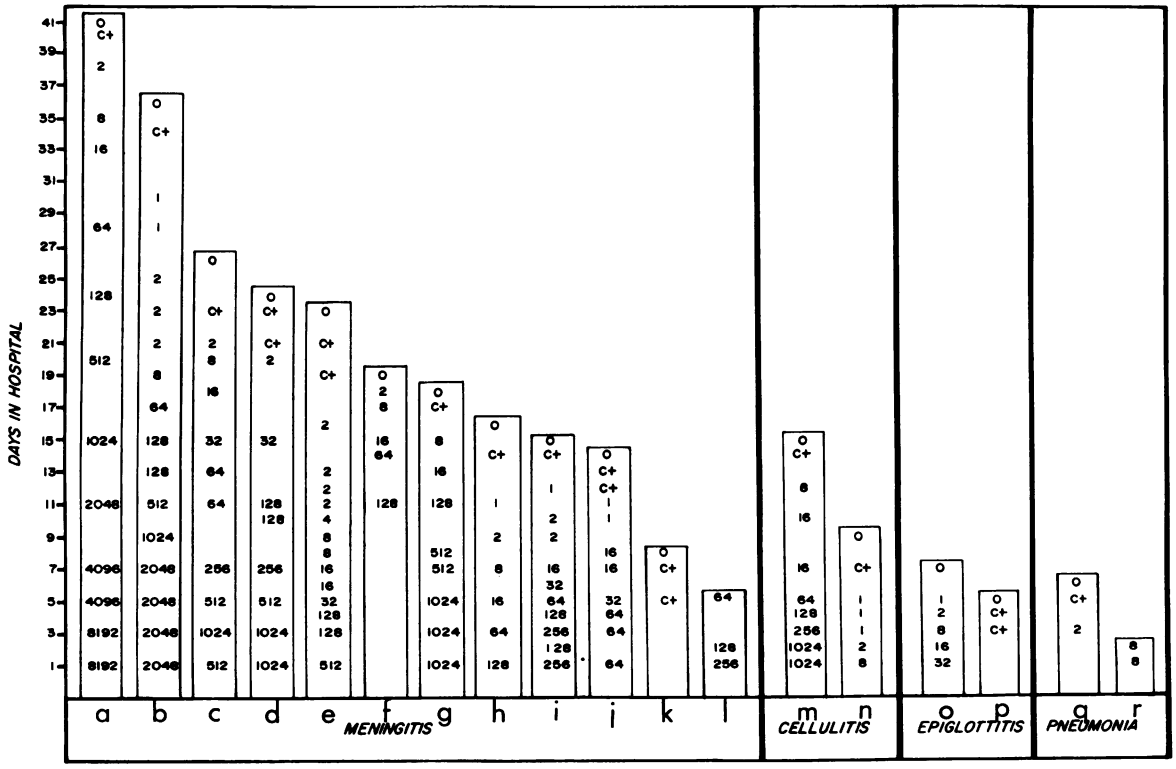

FIG. 1. Titers of latex agglutination tests for *H. influenzae* type b antigen in serial urinary specimens from patients with *H. influenzae* type b infections. The numbers in the vertical columns are the reciprocals of the urinary latex titers. C+ signifies a positive test obtained only after concentration of high-molecular-weight compounds in urine by Amicon filtration. A negative result after Amicon filtration is indicated by 0. Patient l expired and patient r was discharged before their latex agglutination tests became negative.

LITERATURE CITED

1. **Bradshaw, M. W., R. Schneerson, J. C. Parke, Jr., and J. B. Robbins.** 1971. Bacterial antigens cross-reactive with the capsular polysaccharide of Haemophilus influenzae type b. Lancet **i**:1095–1097.
2. **Burdash, N. M., K. A. Smith, and A. L. Walburn.** 1982. Rapid detection of *Haemophilus influenzae* type b in cerebrospinal fluid by commercial coagglutination and latex agglutination kits. Eur. J. Clin. Microbiol. **1**:131–133.
3. **Collins, J. K., and M. T. Kelley.** 1983. Comparison of Phadebact coagglutination, Bactogen latex agglutination, and counterimmunoelectrophoresis for detection of *Haemophilus influenzae* type b antigens in cerebrospinal fluid. J. Clin Microbiol. **17**:1005–1008.
4. **Daum, R. S., G. R. Siber, J. S. Kamon, and R. R. Russell.** 1982. Evaluation of a commercial latex particle agglutination test for rapid diagnosis of *Haemophilus influenzae* type b infection. Pediatrics **69**:466–471.
5. **Kaldor, J., R. Asznowicz, and B. Dwyer.** 1978. *Haemophilus influenzae* type b antigenuria in children. J. Clin. Pathol. **32**:538–541.
6. **Marcon, M. J., A. C. Hamoudi, and H. J. Cannon.** 1984. Comparative laboratory evaluation of three antigen detection methods for diagnosis of *Haemophilus influenzae* type b disease. J. Clin. Microbiol. **19**:333–337.
7. **Schneerson, R., M. Bradshaw, J. K. Whisnant, R. L. Myerowitz, J. C. Parke, Jr., and J. B. Robbins.** 1972. An *Escherichia coli* antigen cross-reactive with the capsular polysaccharide of *Haemophilus influenzae* type b: occurrence among known serotypes, and immunochemical and biologic properties of *E. coli* antisera toward *H. influenzae* type b. J. Immunol. **108**:1551–1562.
8. **Shaw, E. D., R. J. Darker, W. E. Feldman, B. M. Gray, L. L. Pifer, and G. B. Scott.** 1982. Clinical studies of a new latex particle agglutination test for detection of *Haemophilus influenzae* type b polyribose phosphate antigen in serum, cerebrospinal fluid, and urine. J. Clin. Microbiol. **15**:1153–1156.
9. **Thirumoorthi, M. C., and A. S. Dajani.** 1979. Comparison of staphylococcal coagglutination, latex agglutination, and counterimmunoelectrophoresis for bacterial antigen detection. J. Clin. Microbiol. **9**:28–32.
10. **Welch, D. F., and D. Hensel.** 1982. Evaluation of Bactogen and Phadebact for detection of *Haemophilus influenzae* type b antigen in cerebrospinal fluid. J. Clin. Microbiol. **16**:905–908.