JOURNAL OF CLINICAL MICROBIOLOGY, June 1996, p. 1563–1564 Vol. 34, No. 6
0095-1137/96/$04.00+0

Extended Incubation of Culture Plates Improves Recovery of *Bordetella* spp.

GARY KATZKO,[1] MARIANNA HOFMEISTER,[1] AND DEIRDRE CHURCH[1,2,3,4]*

Department of Clinical Laboratories (Microbiology), Alberta Children's Hospital,[1] and Departments of Microbiology and Infectious Diseases,[2] Medicine,[3] and Pediatrics,[4] University of Calgary, Calgary, Alberta, Canada

Received 27 December 1995/Returned for modification 30 January 1996/Accepted 25 March 1996

Extended incubation of culture plates was studied to see if the recovery of *Bordetella* spp. from nasopharyngeal swabs could be improved. Forty-eight *Bordetella* isolates were recovered from 103 children (overall positive-culture rate, 46.6%) who met the clinical case definition of pertussis. Seven of 44 (16%) *B. pertussis* isolates and 2 of 4 (50%) *B. parapertussis* isolates were recovered only after extended incubation of nasopharyngeal cultures up to 12 days.

Pertussis (whooping cough) is a highly contagious, acute infection of the respiratory tract, caused primarily by *Bordetella pertussis* and more rarely by *Bordetella parapertussis*, that is characterized by severe paroxysms of coughing, with or without a classic inspiratory whoop. Although anyone may be infected, young children under 1 year of age have the most serious illness and complication rates (2, 10). Over the past several years, pertussis has been epidemic in the southern Alberta region. Confirmation of a pertussis case in our area had relied on the direct cultivation of *Bordetella* spp. from nasopharyngeal (NP) swabs onto selective medium (i.e., Regan-Lowe) for up to 7 days of incubation. Since molecular methods were not readily available to us, the efficacy of extended culture incubation, including enrichment methods, was studied to see if the recovery of *Bordetella* spp. could be improved.

The Alberta Children's Hospital is a 142-bed primary, secondary, and tertiary regional referral center in Calgary, Alberta, Canada, that is also affiliated with the Faculty of Medicine, University of Calgary. The hospital serves a population base of 1.2 million people and sees children referred there from southern Alberta, southwestern British Columbia, and southeastern Saskatchewan. Approximately 6,700 children are hospitalized each year, while 36,000 children are seen through the emergency department and another 60,000 children visit various subspecialty clinics and physicians' offices.

Children were reported to public health authorities (i.e., Calgary Health Services) even if a clinical diagnosis of pertussis had not been confirmed by a positive NP culture when the following symptoms were present: (i) paroxysmal cough of any duration, (ii) cough with an inspiratory whoop, and/or (iii) cough ending with vomiting or gagging or associated with apnea for no other known cause. During the study period, a total of 172 children were reported from our institution to Calgary Health Services on the basis of clinical symptoms. However, only 103 of these children had NP swabs sent to the laboratory for culture confirmation and were enrolled in the study.

Calcium alginate NP swabs were collected, immediately placed in a charcoal-blood transport medium, and delivered to the laboratory for immediate processing within 30 min of collection. All children had a single NP swab done, and follow-up NP cultures were not performed. All NP swabs received by the laboratory between 15 July 1994 and 31 March 1995 were evaluated in this study. NP swabs were inoculated onto two charcoal agar CM119 plates (Oxoid Ltd., London, England) supplemented with 5% defibrinated horse blood, one of which contained 40 μl of cephalexin per ml. Plates were continuously incubated at 35°C in a humid environment for 3 days and then examined daily for up to 14 days of incubation for the growth of suspicious colonies. The charcoal-blood transport medium (i.e., half-strength charcoal agar with the same blood and cephalexin concentrations as outlined above) was incubated for 2 days and then subcultured and processed in a similar fashion. All colonies with typical morphology were examined by Gram staining, and those with a characteristic gram-negative appearance were confirmed by direct fluorescent antibody staining and slide agglutination testing (Difco Laboratories, Detroit, Mich.) according to the manufacturer's instructions.

A total of 48 positive *Bordetella* cultures were found from 103 children suspected of having pertussis, an overall recovery rate of 46.6%. Of the 48 positive cultures, 44 (92%) were confirmed to be *B. pertussis*, while the other 4 (8%) isolates were *B. parapertussis*. Figure 1 displays the range of incubation days required for the detection of *Bordetella* spp. from both primary medium types as well as the total number of positive cultures which occurred daily from each medium type (i.e., a total of 38 isolates grew on charcoal medium without antibiotics compared with 41 that grew on charcoal medium with cephalexin). Most of the *Bordetella* isolates grew on both primary plates (34 of 48 isolates [71%]). A smaller number of isolates were recovered only on charcoal primary plates without antibiotics (4 of 48 isolates [8%]) or on charcoal medium with cephalexin (7 of 48 isolates [14.5%]). Enrichment cultures recovered only 21 of 48 (44%) isolates, including 3 of 48 (6.3%) that did not grow on either primary medium. The recovery rate of *Bordetella* spp. was substantially enhanced by extending the incubation of plated primary cultures beyond the previous routine of 7 days to up to 14 days (total). If cultures had been routinely terminated after 5 days of incubation, 14 of 45 (31%) isolates would have been missed. If cultures had been terminated after 7 days of incubation, 8 of 45 (18%) isolates would have been missed. No isolates were recovered after 12 days of incubation.

In the previous season when culture plates were incubated only for 7 days, we were able to recover only 30 *Bordetella* isolates from 109 symptomatic children, an overall positive-

* Corresponding author. Mailing address: Department of Microbiology, Alberta Children's Hospital, 1820 Richmond Rd. S.W., Calgary, Alta. T2T 5C7. Phone: (403) 229-7387. Fax: (403) 229-7673.

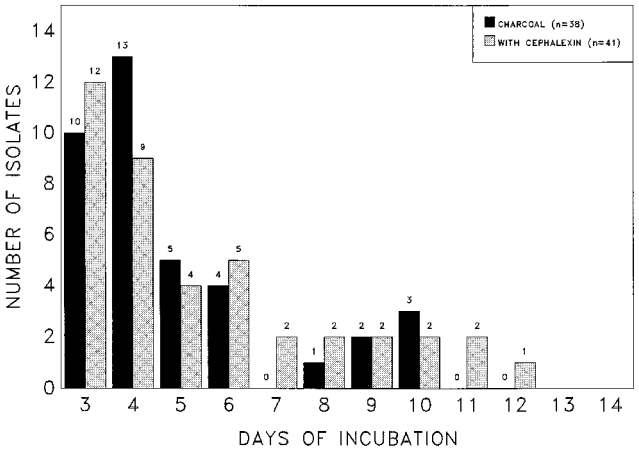

FIG. 1. Recovery of *Bordetella* spp. on charcoal and charcoal medium with cephalexin during a 14-day incubation period.

culture rate of 27.5%. This study demonstrates that the recovery rate of *Bordetella* spp. from NP cultures can be substantially improved (79% increase in positive-culture rate compared with that of the previous year) by extending the plate incubation time. Our routine procedure now includes the incubation of all *Bordetella* NP cultures for 12 days. The primary culture plates are inspected daily for suspicious colonies after an initial 3-day start-up incubation period in which the plates are not taken out of a humid environment. Charcoal medium containing cephalexin is used in addition to charcoal medium without antibiotics since recovery was also enhanced by using selective plates. The use of the charcoal-cephalexin medium increased the identification of *Bordetella* spp. during the extended incubation period when the growth of commensal respiratory flora began to obscure the growth of pertussis isolates on the nonselective medium. The utility of inoculating more than a single primary medium when culturing for *Bordetella* spp. has been previously well documented (3, 4, 8, 9, 11, 13). Although three *Bordetella* isolates were recovered only after enrichment, the increased yield may be outweighed by the overall lack of sensitivity of this method in our study and by the additional associated costs. Enrichment should be individually evaluated in laboratories performing *Bordetella* cultures since previous studies have suggested that it may enhance recovery (11, 14).

Although molecular methodologies (3, 5, 7, 15) and serology (1, 3, 6, 12) may eventually supplement or even supplant routine culturing for *Bordetella* spp., many clinical laboratories currently cannot readily access these newer technologies. Although the recovery of *Bordetella* spp. does not impact clinical management decisions, it is important to evaluate and enhance culture methods not only for epidemiological reasons but also to determine the overall clinical sensitivities of newer test methods (i.e., molecular methods and serological assays) as they become more available to clinical diagnostic laboratories.

The technologists in the microbiology laboratory at the Alberta Children's Hospital deserve thanks for their assistance with this study.

REFERENCES

1. **Conway, S. P., A. H. Balfour, and H. Ross.** 1988. Serologic diagnosis of whooping cough by enzyme-linked immunosorbent assay. Pediatr. Infect. Dis. J. **7:**570–574.
2. **Feigin, R. D., and J. D. Cherry.** 1992. Pertussis, p. 1208–1218. *In* R. D. Feigin and J. D. Cherry (ed.), Textbook of pediatric infectious diseases. W. B. Saunders Co., Philadelphia.
3. **Friedman, R. L.** 1988. Pertussis: the disease and new diagnostic methods. Clin. Microbiol. Rev. **1:**365–376.
4. **Gilchrist, M. J. R.** 1990. Laboratory diagnosis of pertussis. Clin. Microbiol. Newsl. **12:**49–53.
5. **Glare, E. M., J. C. Paton, R. R. Premier, A. J. Lawrence, and I. T. Nisbet.** 1990. Analysis of a repetitive DNA sequence from *Bordetella pertussis* and its application to the diagnosis of pertussis using the polymerase chain reaction. J. Clin. Microbiol. **28:**1982–1987.
6. **Halperin, S. A., R. Bertolussi, and A. J. Wort.** 1989. Evaluation of culture, immunofluorescence, and serology for the diagnosis of pertussis. J. Clin. Microbiol. **27:**752–757.
7. **He, Q., J. Mertsola, H. Soini, and M. K. Viljanen.** 1994. Sensitive and specific polymerase chain reaction assays for detection of *Bordetella pertussis* in nasopharyngeal specimens. J. Pediatr. **124:**421–426.
8. **Hoppe, J. E.** 1988. Methods for isolation of *Bordetella pertussis* from patients with whooping cough. Eur. J. Clin. Microbiol. **7:**616–620.
9. **Kurzynski, T. A., D. M. Boehm, J. A. Rott-Petri, R. F. Schell, and P. E. Allison.** 1988. Comparison of modified Bordet-Gengou and modified Regan-Lowe media for the isolation of *Bordetella pertussis* and *Bordetella parapertussis*. J. Clin. Microbiol. **26:**2661–2663.
10. **Manclark, C. R., and J. L. Cowell.** 1984. Pertussis, p. 69–106. *In* R. Germanier (ed.), Bacterial vaccines. Academic Press, Inc., New York.
11. **Marcon, M. J.** 1995. *Bordetella*, p. 566–573. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
12. **Mertsola, J., O. Ruuskanen, T. Kuroren, O. Meurman, and M. K. Viljanen.** 1990. Serologic diagnosis of pertussis: evaluation of pertussis toxin and other antigens in enzyme-linked immunosorbent assays. J. Infect. Dis. **161:**966–971.
13. **Onorato, I. M., and S. G. F. Wassilak.** 1987. Laboratory diagnosis of pertussis: the state of the art. Pediatr. Infect. Dis. J. **6:**145–151.
14. **Regan, J., and F. Lowe.** 1977. Enrichment medium for the isolation of *Bordetella*. J. Clin. Microbiol. **6:**303–309.
15. **Van der Zee, A., C. Agterberg, M. Peeters, J. Schellekens, and F. R. Mooi.** 1993. Polymerase chain reaction assay for pertussis: simultaneous detection and discrimination of *Bordetella pertussis* and *Bordetella parapertussis*. J. Clin. Microbiol. **31:**2134–2140.