JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1993, p. 50–52
0095-1137/93/010050-03$02.00/0

Vol. 31, No. 1

Comparison of Nasopharyngeal Aspirates with Swabs for Culture of *Bordetella pertussis*

HANS O. HALLANDER,[1]* ELISABET REIZENSTEIN,[1] BERIT RENEMAR,[2] GUN RASMUSON,[2] LENA MARDIN,[1] AND PATRICK OLIN[2]

Department of Bacteriology, National Bacteriological Laboratory, 105 21 Stockholm,[1] and Department of Paediatrics, Karolinska Institute, Sachs' Children's Hospital, Stockholm,[2] Sweden

Received 17 July 1992/Accepted 22 October 1992

Nasopharyngeal samples were collected from 117 children by aspiration from one nostril and by swab from the other one and cultured for *Bordetella pertussis*. Among 33 culture-positive specimens, there were 30 positive aspirates and 26 positive swab specimens. Aspirates are easily divided and saved for investigations with other assays which may further improve diagnostic sensitivity. As the aspiration technique was also preferred by nurses and parents, it was recommended and chosen for a planned pertussis vaccine efficacy trial to take place in Sweden from 1992 to 1995.

Although culture is the standard method for diagnosis of pertussis infections, recent vaccine efficacy trials have prompted a reconsideration of culture as a single marker for case definition. One problem is that the sensitivity of culture is only about 50% compared with serologic testing (2, 3, 9). To some extent, however, this is probably because the concentration of bacteria has started to decrease when the typical symptoms begin. The fact that repeated cultures in clinically suspected cases of pertussis increased the number of positive cultures by 25% also supports suspicions that sampling often is inappropriate (3). The collection procedure (1, 7, 10) and swab material (1, 6, 13) are factors that have a great impact on the recovery of *Bordetella pertussis*. Although it is regarded as less practical than nasopharyngeal swabbing, sampling by aspiration has been advocated by some authors (1, 14). The continuing controversy over the appropriate temperature for transport (5, 8) also emphasizes the advantage of bedside plating of samples. In this study, collection of samples by nasopharyngeal aspiration was compared with swabbing the nasopharynx of the same patient with the aim of determining the optimal diagnostic method for a pertussis vaccine efficacy trial to take place in Sweden from 1992 to 1995.

MATERIALS AND METHODS

Clinical material. Samples were collected by swabbing as well as aspiration from two groups of children.

The first part of the study took place during the spring of 1991 and involved 100 children, all below 4 years of age (median age, 2.1 years), with suspected whooping cough. From this group, 51 swabs were collected for culture, and the remaining swabs were collected for alternative assays (data not shown). Two specially trained nurses were responsible for the sampling. For all children, a questionnaire concerning preference for swab sampling versus aspiration was completed independently by study nurses and parents.

The second study involved 66 suspected cases of whooping cough during the spring of 1992. The median age of the patients was 2.5 years (range, <1 to 45 years). In this study, performed by a team of three specially trained nurses, the aspiration technique was slightly modified and no clinical data were collected.

Collection of samples by aspiration. Aspirates were collected before swabbing was done. A Bardic feeding tube (infant size) was connected via a mucus trap (kit for tracheal suction; Nunc, Roskilde, Denmark) to a hand vacuum pump with tubing (Nalgene Company, Rochester, N.Y.). The end of the catheter was inserted into one nostril to the posterior pharynx along the floor of the nasopharynx.

For the first group of patients, suction was applied when the posterior pharynx was reached and was maintained as the catheter was slowly withdrawn to the middle of the nasal cavity. Videotapes recording the collection, however, indicated that in some cases the catheter was not properly inserted into the posterior pharynx. Because of these observations, the collection procedure was slightly modified in the second study. The procedure then was to apply suction twice with the hand vacuum pump when the posterior pharynx was reached.

The catheter was flushed by aspirating 1.0 ml of phosphate-buffered saline through the catheter into the trap. The tip of the catheter was dipped and stirred in the aspirate, inserted into the transport medium, and cut. Direct plating of aspirates was not performed because of technical difficulties.

Collection of samples by swabs. From the other nostril, nasopharyngeal specimens were taken with Dacron swabs (MW 160 pernasal swabs; Medical Wire & Equipment, Corsham, Wiltshire, United Kingdom) tested for nontoxicity to *B. pertussis*. These swabs were passed as far posterior into the nasopharynx as possible, rotated, and left for 5 to 15 s to moisten. The samples were inoculated onto isolation medium plates (see below) in the patients' homes and then inserted into the transport-and-enrichment medium.

Culture media. (i) Isolation medium. Isolation medium plates were prepared by the method of Regan and Lowe (11) with selective charcoal agar (4.6%) plates (model CM 119; lot number 34046048; Oxoid, Basingstoke, United Kingdom) with 10% defibrinated horse blood and 40 mg of cephalexin (Eli Lilly and Company, Indianapolis, Ind.) per liter. Each plate was stored in a plastic bag at 4 to 8°C. Each agar preparation was controlled by performing a viable-cell count dilution series with a *B. pertussis* strain (ATCC 9797).

(ii) Transport-and-enrichment medium. Transport-and-enrichment medium was prepared as described above for

* Corresponding author.

TABLE 1. Preference data for sampling methods in study 1

Individuals surveyed	No. with the following preference (n = 100):				No. to whom question was not posed
	Swab	Aspirate	Equal	No opinion	
Children	6	8	3	2	81
Parents	15	63	11	7	4
Nurses	16	51	20	9	4

isolation medium but with only half the agar concentration. Aliquots (2.5 ml) were dispensed into screw-cap glass tubes (45 by 15 mm).

Processing at the bacteriological laboratory. Aspirates were mechanically homogenized with a Vortex-Genie mixer (Scientific Industries) for at least 30 s, and 100 μl was inoculated onto charcoal agar plates. The plates were incubated for 7 days at 35 to 37°C in plastic bags together with those inoculated at the bedside. Transport-and-enrichment medium tubes were incubated at 35 to 37°C for 72 h, subcultured by carefully rotating a fresh swab at the inoculation site in the medium, and then plated on charcoal agar, which was incubated for at least four additional days.

Identification of *B. pertussis*. Suspected *B. pertussis* colonies were identified primarily by slide agglutination with specific antisera to *B. pertussis* and *B. parapertussis* (batches 785820 and 764668, respectively; Difco, Detroit, Mich.). Identifications of all isolates were biochemically verified by testing for catalase, oxidase, urea, nitrate reduction, pigmentation on tyrosine agar, and growth on blood agar.

RESULTS

B. pertussis was isolated in 33 of the 117 cases from swabs, aspirates, or both. *B. pertussis* was isolated from 30 aspirates (91%) and from 26 swabs (79%). *B. parapertussis* was not found in this material.

Among 19 culture-positive cases in the first study, 17 were identified from aspirates and 16 were identified from swabs. In the second study, after modification of the aspiration technique, 14 cases were culture positive; among these cases, 13 were identified from aspirates and 10 were identified from swabs. Three aspirates and two swab samples were positive after enrichment only.

Three cases (two in study 1 and one in study 2) were culture negative by the aspiration technique but positive by the swab technique. In two of these, fewer than 10 colonies were found on the plates. The third patient had taken four doses of erythromycin.

When the culture results were related to the time interval between onset of cough and collection of specimens (study 1), it was found that two of the six culture-positive specimens collected within 1 week were positive by the swab technique but negative by the aspiration technique. After 15 days, all samples were positive by both techniques.

Although the difference was not significant, aspiration provided a higher yield of bacteria than swabbing. Among 27 culture-positive aspirates, 20 (74%) yielded ≥50 colonies on the plate, whereas only 15 of 25 (60%) swab samples yielded ≥50 colonies on the plate.

Sampling technique preference. The aspiration technique was well tolerated by the children and subjectively preferred by most of the parents (Table 1). Only a few of the children were old enough to state a preference for either of the sampling techniques. The nurses preferred aspiration for technical reasons as well as on the basis of their perception of the children's preferences.

DISCUSSION

Using culture for case definition, e.g., in vaccine trials, is insufficient because of its low sensitivity. An additional problem is that the sensitivity of culture for vaccinated individuals differs from that for unvaccinated individuals (3). Therefore, all possible ways of improving culture results should be investigated.

Direct plating onto selective medium in the home together with enrichment procedures increases the frequency of positive cultures (1, 4, 15). An earlier study showed that using enrichment tubes and plates together increased the number of positive cultures by 14% compared with using only plates (3). In the present study, the corresponding increases were 8% for swabs and 11% for aspirates.

At a World Health Organization working group meeting on case definitions of pertussis in Geneva, Switzerland, in January 1991, laboratory research priority was given to tests for detection of *B. pertussis* organisms or the products of such organisms by such methods as polymerase chain reaction, (PCR), DNA hybridization, and antigen detection by enzyme immunoassay. However, a successful outcome of such efforts as well as of the culture procedure is dependent on adequate collection procedures and on the ability to divide or save samples, as may be done with aspirates.

An objective evaluation of the two sampling techniques, however, is hampered by sampling difficulties and the low prevalence of pertussis. In this study, nurses employed for a planned pertussis vaccine efficacy trial succeeded in collecting specimens by aspiration as well as swabbing from 117 children. Although the difference was not significant, aspiration yielded more positive results than swabbing. However, some samples were positive by the swab technique but negative by aspiration. One explanation may be that collection was done by inserting the swab and the aspiration tube through different nostrils. Another problem is the practical difficulties linked with obtaining samples from children; these difficulties resulted in aspiration being the preferred technique and thus being done before swab sampling. This may also have introduced a favorable bias for aspiration.

The occurrence of swab-positive but aspirate-negative samples early in disease may be due to the relatively scanty secretion of mucus in the early stages.

The potential to improve diagnostic sensitivity by the aspiration technique was further demonstrated by the fact that in addition to the 30 of 33 aspirates that were positive by culture, another 7 were positive by PCR (unpublished data). Also, previous DNA hybridization studies indicated that at least 50% of the bacteria were attached to swabs and could not be recovered (12).

In conclusion, aspiration resulted in a sensitivity in culture comparable to or higher than that of swab sampling and facilitated the use of other assays. The aspiration technique was also preferred by the majority of nurses and parents. Therefore, the aspiration technique was recommended as a routine procedure for a Swedish pertussis vaccine efficacy trial to be conducted from 1992 to 1995.

ACKNOWLEDGMENTS

This work was financially supported by the National Institute of Allergy and Infectious Diseases (contract NO1-A1-15125).

We thank Jann Storsaeter for his valuable advice. The collection of specimens and data by study nurses and the participation of parents and children in this study are gratefully acknowledged.

REFERENCES

1. **Gilchrist, M. J. R.** 1990. Laboratory diagnosis of pertussis. Clin. Microbiol. Newsl. **12**:49–53.
2. **Granström, G., B. Wretlind, and M. Granström.** 1991. Diagnostic value of clinical and bacteriological findings in pertussis. J. Infect. **22**:17–26.
3. **Hallander, H. O., J. Storsaeter, and R. Möllby.** 1991. Evaluation of serology and nasopharyngeal cultures for diagnosis of pertussis in a vaccine efficacy trial. J. Infect. Dis. **163**:1046–1054.
4. **Hoppe, J. E.** 1988. Methods for isolation of *Bordetella pertussis* from patients with whooping cough. Eur. J. Clin. Microbiol. **7**:616–620.
5. **Hoppe, J. E.** 1989. Recovery of *Bordetella pertussis* from nasopharyngeal swabs. J. Clin. Microbiol. **27**:595–596.
6. **Hoppe, J. E., and A. Weiss.** 1987. Recovery of *Bordetella pertussis* from four kinds of swabs. Eur. J. Clin. Microbiol. **6**:203–205.
7. **Lautrop, H., and B. W. Lacey.** 1960. Laboratory diagnosis of whooping cough or *Bordetella* infections. Bull. W.H.O. **23**:13–35.
8. **Morrill, W. E., J. M. Barbaree, B. S. Fields, G. N. Sanden, and W. T. Martin.** 1988. Effects of transport temperature and medium on recovery of *Bordetella pertussis* from nasopharyngeal swabs. J. Clin. Microbiol. **26**:1814–1817.
9. **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.
10. **Marcon, M. J., A. C. Hamoudi, H. J. Cannon, and M. M. Hribar.** 1987. Comparison of throat and nasopharyngeal swab specimens for culture diagnosis of *Bordetella pertussis* infection. J. Clin. Microbiol. **25**:1109–1110.
11. **Regan, J., and F. Lowe.** 1977. Enrichment medium for the isolation of *Bordetella*. J. Clin. Microbiol. **6**:303–309.
12. **Reizenstein, E., E. Morfeldt, G. Gilljam, H. O. Hallander, and S. Löfdahl.** 1990. A DNA hybridization test for detection of *Bordetella* in nasopharyngeal specimens. Mol. Cell. Probes **4**:211–221.
13. **Ross, P. W., and C. G. Cumming.** 1981. Isolation of *Bordetella pertussis* from swabs. Br. Med. J. **283**:403–404.
14. **Wort, A. J.** 1983. Bacteriological diagnosis of pertussis. Lancet **i**:766.
15. **Young, S. A., G. L. Anderson, and P. D. Mitchell.** 1987. Laboratory observations during an outbreak of pertussis. Clin. Microbiol. Newsl. **9**:176–179.