Bordetella holmesii-Like Organisms Isolated from Massachusetts Patients with Pertussis-Like Symptoms

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We isolated *Bordetella holmesii*, generally associated with septicemia in patients with underlying conditions, from nasopharyngeal specimens of otherwise healthy young persons with a cough. The proportion of *B. holmesii*-positive specimens submitted to the Massachusetts State Laboratory Institute increased from 1995 to 1998.

Bordetella holmesii is a recently described gram-negative, asaccharolytic, nonoxidizing, soluble, brown-pigment-producing rod previously known as CDC nonoxidizer group 2 (NO-2) (1). This group consists of 15 closely related, biochemically similar strains of fastidious nonmotile bacteria isolated from human blood cultures. In establishing NO-2 as a species, Weyant et al. (1) performed 16S rRNA sequencing of one NO-2 strain and the type strains of *B. pertussis, B. parapertussis, B. bronchiseptica,* and *B. avium*. They found a high degree of homology among them (≥98% over 1,525 bases) and confirmed a close relatedness between NO-2 and *Bordetella* species by DNA relatedness studies (hydroxyapatite method). Biochemically, the lack of oxidase activity and the production of a brown soluble pigment differentiate *B. holmesii* from *B. pertussis, B. bronchiseptica,* and *B. avium*; the lack of urease activity differentiates it from *B. parapertussis* (1).

Unlike *B. pertussis*, which causes whooping cough, *B. holmesii* has been associated most often with septicemia in patients with underlying conditions (1-4). It also has been isolated from sputum from one patient with respiratory symptoms (3). Van den Akker (5) suggested that the difference in lipopolysaccharide expression (important in bacterial pathogenesis) between the closely related *B. pertussis* and *B. holmesii* might help explain their observed difference in propensity to infect respiratory tract epithelium verses causing opportunistic bacteremia.

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In Massachusetts, however, we have seen *B. holmesii* associated with a different clinical picture. From January 1995 (when the article describing *B. holmesii* [1] was published) through December 1998, the Massachusetts State Laboratory Institute (SLI) isolated *B. holmesii* from 34 clinical specimens: 33 nasopharyngeal specimens from patients suspected of having pertussis and one blood culture specimen from a 45-year-old patient with septicemia. Of the 33 patients with respiratory symptoms, 30 (91%) were 11 to 29 years old, 1 (3%) was an infant, and 2 (6%) were 10 years old; most were otherwise healthy.

B. holmesii is confirmed by its biochemical patterns and cellular fatty acid analysis or the DNA transformation test will definitively separate *B. holmesii* from *Acinetobacter*, neither procedure is performed at SLI. Three of the initial isolates were sent to the Centers for Disease Control and Prevention, Atlanta, Georgia, for definitive identification and were confirmed as *B. holmesii* by cellular fatty acid analysis. The remaining 31 isolates were biochemically and morphologically identical to those.

B. holmesii–positive nasopharyngeal specimens have increased both in absolute number and as a percentage of the total nasopharyngeal specimens processed at SLI (Table). The number rose from 3 (0.1% of total specimens submitted for pertussis culture) in 1995, to 6 (0.2%) in 1996, Table. *Bordetella* species isolated from nasopharyngeal (NP) specimens at the Massachusetts State Laboratory Institute, 1994–1998

	1994		1995		1996		1997		1998	
	No.	%	No.	%	No.	%	No.	%	No.	%
B. pertussis	75	4.2	140	5.8	325	8.9	132	5.6	165	6.6
B. parapertussis	7	0.4	20	0.8	32	0.9	11	0.5	NA	NA
B. holmesii	0	0	3[a]	0.1	6	0.2	9	0.4	15	0.6
Total NP specimens reported	1,792		2,399		3,653		2,375		2,508	

[a]Does not include the one case of *B. holmesii* isolated from blood.

to 9 (0.4%) in 1997, and to 15 (0.6%) in 1998. A chi-square analysis for linear trend of proportions from 1995 to 1998 found the trend significant (chi-square = 11.6, $p < .001$), possibly indicating a rise in prevalence. (When such an analysis was applied to the proportion of nasopharyngeal specimens testing positive for pertussis during the same period, no trend was apparent ([chi-square = 0.8, $p = .36$].) A growing awareness of the species by laboratory personnel may be contributing to the observed increase.

To ascertain what symptoms were associated with *B. holmesii* isolation, we investigated the 23 cases identified in the 24 months from January 1997 through December 1998. We called providers and patients for disease histories and demographic information. Pertussis case report forms, modified to include more possible symptoms and underlying conditions, were used to record the information collected in the interviews.

Nineteen (83%) of the 23 *B. holmesii*-positive cases were in adolescents (11 to 19 years), 2 (9%) in young adults (20 and 29 years), 1 (4%) in a 10-year-old child, and 1 (4%) in an infant. All had cough. In addition, 14 (61%) had paroxysms, 2 (9%) had whoop, and 6 (26%) had posttussive vomiting. No other symptoms were identified by patients or providers. Fourteen (61%) of the 23 had no underlying conditions, 8 (35%) had minor conditions such as occasional asthma or allergies, and 1 (4%) had chronic fatigue.

The fact that cultures were taken from 20 (87%) of the 23 case-patients within 14 days of cough onset generally excluded convalescent-stage pertussis as a cause of symptoms. However, *B. pertussis* had been confirmed in a 14-year-old girl, who had occasional asthma, 3 months before *B. holmesii* was confirmed—she received a reculture because of a persistent cough that had not resolved since the original infection. She had had paroxysms and vomiting associated with the pertussis infection but no symptoms other than cough at the time *B. holmesii* was isolated. Cultures were taken on the same day from two sisters, 15 and 9 years old, each with cough of fewer than 14 days. *B. holmesii* was culture-confirmed in the 15-year-old; *B. pertussis* was culture-confirmed in the 9-year-old. This raises the question of whether *B. pertussis* and *B. holmesii* might cocirculate.

At least 11 (48%) of the 23 cases were found during active surveillance for pertussis in school and university settings, which may explain the age profile of the cases. Three case-patients, with cough onset dates of April 1, 1997; February 27, 1998; and March 9, 1998, were students at the same university. These cases, though not epidemiologically linked to each other, were in symptomatic contacts of pertussis patients and were cultured as part of an azithromycin efficacy study. At least eight other cases were also in contacts of confirmed patients with pertussis and were detected through active surveillance. No cases were definitively linked. Peak months of cough onset were November and December (8 of the 23 cases), as is true for pertussis in Massachusetts, with a smaller peak in March and April (6 of the 23 cases). The observed peak in November-December may be due to the role of active surveillance for pertussis in ascertaining cases of *B. holmesii* colonization.

The clinical profile of the 21 cases in adolescent and adult patients infected with *B. holmesii* was compared with that of 122 culture-confirmed pertussis cases in patients between 11 and 29 years of age with cough onsets in the same period, i.e., 1997 through 1998. (Relevant clinical information was not available for an additional 42 culture-confirmed pertussis case-patients in this age group.) We did not consider cough duration, because of imprecise data, but rather focused on the presence or absence of three classic pertussis symptoms: paroxysms of cough, whoop, and posttussive vomiting. Cases were categorized as patients with 0, 1, or 2 to 3 of these symptoms. (No separate category for three symptoms was used due to a cell size of 0 in the case of *B. holmesii*.) On applying the chi-square test for independent proportions, we found *B. holmesii* infection milder (i.e., accompanied by fewer of the above three pertussis symptoms) than *B. pertussis* infection (chi-square = 10, $p < .01$).

To rule out the possibility that the difference was due to more frequent cultures for severe than for milder pertussis cases, we compared the 21 adolescent and adult *B. holmesii* patients with the 577 SLI–serology-positive pertussis patients 11 to 29 years of age with cough onsets in 1997 or 1998 for whom sufficient clinical data were available. The SLI pertussis serology test is a single-serum enzyme-linked immunosorbent assay for immunoglobulin G to pertussis toxin, available since 1987. The assay is for use in persons ≥ 11 years of age and is optimally sensitive at 2 to 8 weeks after cough onset. By the same methods as for the previous comparison, we found that the *B. holmesii* cases were milder at a higher level of significance (chi-square = 69, $p < 10^{-8}$).

Without knowing the prevalence of *B. holmesii* carriage in asymptomatic persons, we cannot say with certainty that *B. holmesii* is the causative agent for the respiratory symptoms of the patients from whom it was isolated. Approximately half the cases were discovered through active surveillance for pertussis. This, together with the fact that the symptoms associated with *B. holmesii* were relatively mild, suggests that the organism may not have been causing disease. On the other hand, *B. holmesii* may be the etiologic agent, given that it is closely related to *B. pertussis* and the associated symptoms (like those of *B. parapertussis)* are similar. *B. pertussis* is the only *Bordetella* species known to produce pertussis toxin, although *B. parapertussis* and *B. bronchiseptica* have silent copies of the toxin gene (6). We do not know whether *B. holmesii* has the toxin gene. However, since *B. parapertussis* can cause disease (albeit not as severe as *B. pertussis* [7]), the presence of pertussis toxin is not necessary for the development of symptoms.

Continued investigation, including conducting diagnostic tests for agents such as *Chlamydia* and *Mycoplasma* and culturing symptomatic and asymptomatic contacts, is warranted to ascertain the degree to which *B. holmesii* is pathogenic in the respiratory system and contagious. If it is contagious, antibiotic susceptibility testing is also needed. *B. holmesii* is susceptible to some 15 antibiotics of a variety of classes (2,3), but whether erythromycin, the drug of choice for pertussis, is effective is not known. SLI is establishing routine erythromycin susceptibility testing of *B. pertussis* and will also test *B. holmesii* isolates.

Acknowledgment

We thank Colin D. Marchant for early suggestions regarding data analysis.

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